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Vol. I FEBRUARY, 1916 No. 5

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Fig. 1.—To show the contrast between the constricted vessels still connected with the vaso-constrictor center (left ear) and the control dilated vessels that have been disconnected from that center (right ear). Drawing made late in the experiment when the animal was apparently in a state of deep shock. The strong vaso-constriction in the left ear was replaced by a wide dilatation as soon as the connection of this ear with the vasomotor center was severed. (See Protocol V.)

The Journal of Laboratory and Clinical Medicine

VOL. I. ST. LOUIS, FEBRUARY, 1916 NO. 5.

ORIGINAL ARTICLES

ON THE CONDITION OF THE VASO-CONSTRICTOR CENTER DURING THE DEVELOPMENT OF SHOCK*

BY MAJOR G. SEELIG, M.D., AND DON R. JOSEPH, M.D., ST. LOUIS, MO.

SO many reviews of the literature on surgical shock have appeared that we deem it unnecessary to enter here into such a discussion. The term is in itself too generic and although we make no attempt to define it, we use the term surgical shock to cover that compromise of the animal which results from operative trauma and which is analogous to the compromise that occasionally follows surgical therapeutic procedures on man. Our interest is centered in the view, which has been accepted too generally and upon insufficient evidence, that in surgical shock the *primary* cause of all the other symptoms is a preceding paralysis of the vaso-motor center. Since Porter first attempted to show that the vaso-constrictor center is still in a state of pronounced functional activity after a shock level of blood pressure has been reached, there have been attempts made by several investigators to bring forward evidence bearing upon this point.[1]

PROBLEM.

Our problem was essentially this: Can we obtain any conclusive evidence that, during the development of shock, the vaso-constrictor center at any time loses its activity? If we could show that the center loses its activity very early, it would be reasonable to believe that some, if not many of the other symptoms of shock, are secondary to this failure. If, on the other hand, indisputable

*From the Department of Physiology of St. Louis University School of Medicine.

A preliminary statement of the results of the present communication appeared in the Proc. Soc. Exper. Biol. and Med., 1914, 12, p. 49.

Also a demonstration of the results herein described was given before the Amer. Physiol. Society at its meeting in St. Louis, in Dec., 1914.

[1]Porter, W. T., and Quinby, W. C.: Am. Jour. Physiol., 1914, 10, xii.
Porter, Marks and Swift: Am. Jour. Physiol., 1907, xx, 444.
Porter and Quinby: Am. Jour. Physiol., 1908, xx, 500.
Seelig and Lyon: Jour. Am. Med. Assn., 1909, lii, 45.
Bartlett: Jour. Exper. Med., 1912, 15, 415.
Mann: Bull. Johns Hopkins Hosp., 1914, xxv, 205.

evidence could be obtained that the activity of the vaso-constrictor center persists for a long time and even after many of the other manifestations of shock are present, we could then say definitely that shock is not the result of a primary breakdown of the vaso-motor center but must be due to some other cause or causes.

Our first requirement was a region of blood vessels, the calibre of which could be observed without any operative interference. Second, a region the observations upon which could be controlled by comparing it to its denervated mate upon the other side of the midline, in the same animal.

The retinæ of albino rabbits are non-pigmented and the retinal vessels stand out distinctly. In our early experiments, we tried to denervate the vessels of one retina and after producing shock in the animal, to observe and compare the size of the vessels in the two retinæ. Theoretically this method should be satisfactory, but for practical reasons it was finally discontinued. We need mention here only two of these reasons. First, the origin and course of the vaso-motor supply to the retinal vessels is not perfectly understood; consequently, it might be claimed that the retinal vessels of the supposedly denervated side were not entirely isolated from connection with the vaso-motor center, in which case we should have no control vessels. Second, it is necessary to depend upon the judgment of the ophthalmologist for a comparison of the vessels in the two eyes, thus introducing too many opportunities for error and furnishing us no chance to check our own results by direct observation.

We finally selected the ear vessels of white rabbits. This region seems almost ideal for our purpose. First of all, the vessels can be seen distinctly by anybody. This means that a comparison of the ear vessels can be made by any number of individuals, either trained or untrained. Secondly, the vessels are subjected to no direct operative interference at any time. Thirdly, the course of the vaso-constrictor nerve fibers to the ear vessels has been well worked out[2] and we can be sure therefore that the vessels of the control ear have really been disconnected from the medullary center.

The following is the plan by which we attacked the problem: If the vaso-constrictor nerves to one ear are cut we remove all influence of the vaso-constrictor center over the vessels of that ear. The denervated vessels might then be expected to react more or less passively to changes of pressure within them. If the heart were maintaining a high blood pressure these vessels should become passively dilated. If, on the other hand, the blood pressure were low they should be correspondingly less dilated. In other words, the control vessels of the denervated ear should react passively no matter what the pressure within them. Why do we need such a set of control vessels? Suppose we reduce an animal to a state of shock and find that ear vessels connected with the vaso-constrictor center are empty. This emptyness may be either an active or a passive one. That is, it may be caused by a stream of vaso-constrictor impulses flowing out from the center in the medulla and maintaining in the constricted vessels a tonus sufficiently active to force out the blood, or it may be due to a passive drainage of the blood from these vessels into the great splanchnic reservoir. If we denervate the vessels of one ear, the vessels of that ear—if they respond passively

[2]Meltzer and Meltzer: Am. Jour. Physiol., 1903, ix, 57.

to pressure changes within them—will tell us whether the constriction present in the normal ear is an active or a passive one. Especially is this true if we can, by suitable means, raise temporarily the blood pressure from its low shock level. The denervated vessels should passively dilate in response to this elevation of pressure. The normal ear vessels, if they are receiving no vaso-constrictor impulses should also dilate; if, however, they resist the rise in pressure and remain constricted, we are justified in concluding that the constriction is the result of vaso-constrictor impulses arising outside the vessels, presumably in the medulla.

Furthermore, the ability of the vessels of the normal ear to remain constricted under the temporarily increased blood pressure should be an index of the degree of tonus being maintained by the center in those vessels. For example, if the constriction persists in the normally innervated vessels after the blood pressure has risen to a high point, we must conclude that the center is maintaining a high degree of tonus.

The question of the degree of passive response obtainable from denervated vessels at different intervals after denervation will be discussed a little later.

Experimental Method.

As already stated, white rabbits were used exclusively. The vessels of one ear were denervated by resecting and severing, under ether anesthesia, the auricularis magnus nerve at the base of the pinna and removing the superior cervical sympathetic ganglion on the same side. In no case was the animal reduced to a state of shock immediately following the denervation of the ear. At least 20 hours elapsed between the two operations and in some cases this interval was a number of days in length. The purpose of this delay was to avoid the effects upon the denervated vessels of immediate stimulation of vaso-dilator or vaso-constrictor nerve fibers consequent upon the operative procedures at the time of the denervation. After this interval, the animal was again etherized and reduced to a state of shock. In most cases it was not necessary, nor indeed advisable, to continue the etherization for more than ¾ to 1 hour, for at that time most animals showed only a slight reaction, if any, to sensory stimulation. Tracheotomy was performed and the abdomen opened wide by a long median incision. In a majority of the experiments the adrenals were carefully removed at once upon opening the abdomen. This was done on the assumption that adrenalin thrown into the blood stream during the experiment might possibly interfere with a passive dilatation of the vessels of the denervated ear, and at the same time favor constriction in the vessels of the normal ear, a constriction which would be of peripheral rather than of central origin. Also it was thought that the removal of the adrenals might hasten somewhat the fall of the blood pressure. We are unable to draw any definite conclusions as regards either of the above possibilities since we made no systematic comparison between animals with intact and with excised adrenals. At any rate, the removal of the adrenals, practiced in most of the experiments, with the attendant manipulation of and traction upon the viscera and sympathetic nervous structures may be considered one of the means used to induce shock.

Next the abdominal aorta was exposed for a short distance between the origin of the celiac artery and the diaphragm, and a loose ligature passed around it to facilitate the application of a clamp when desirable. Clamping the aorta at this point was the method used to raise temporarily the blood pressure, in the part of the animal anterior to the diaphragm, when the general blood pressure had fallen to a low level. The reason for raising the blood pressure at this time has already been stated. Tracings of the blood pressure were taken from a mercury manometer connected with a cannula in one axillary artery. As an index to the degree of shock we have relied largely upon the state of the blood pressure; we did not confine ourselves, however, to observations upon this one symptom of shock. The state of the reflexes was used as was also the response to sensory stimulation applied to various regions. We continued the experiment not only until the blood pressure had fallen to a low level but until the reflexes became sluggish or were practically absent, and there was little, if any, response to handling of the abdominal viscera or parietal peritoneum. The rabbit seems to sink to this condition much more readily than do most dogs.

As a second and final test of whether the constriction in the normally innervated vessels was an active or a passive one, we denervated, late in the experiment, the vessels of the normal ear. This was done in some cases by cutting the nerves, in others by abolishing the nerve conductivity through direct application of an ether soaked pledget of cotton to the nerve trunk, or by freezing the nerve at one point with an ethyl chloride spray. We found that, for practical purposes, severing the conductivity of the cervical sympathetic trunk was sufficient to bring out the effect in the "normal" ear vessels and therefore in most cases we did not cut the auricularis magnus nerve. Another reason why we usually left the auricularis magnus intact was that we wished to disturb the animal as little as possible. (The cervical sympathetic is the more easily obtainable of the two nerves that carry a vaso-motor supply to the ears.)

In a few experiments the ears of the animals were photographed during the course of the experiment. For this purpose we used light transmitted through the ears to the camera from an electric arc. In one case a water color drawing was made from life toward the end of the experiment.

Experimental Results.

Twenty-five experiments were performed in this series. The interval between denervation of the blood vessels of one ear and the production of shock varied. In eleven experiments this interval was 20 to 28 hours; in two experiments it was 2 days; in five experiments it was 3 days; in two experiments 4 days; in two experiments 6 days; and finally one experiment each was performed in which the interval was 7, 9 and 12 days respectively. As has already been stated, this interval was allowed in order to secure a proper set of control vessels. It concerns in no way the vessels of the normal ear directly.

On the Suitability of "1," "2," "3" (and more) Day Rabbits for this Kind of Test.

The term "one," "two," or "three" day rabbit will refer to the length of the interval which elapsed between the denervation of the blood vessels of the control ear and the execution of the shock experiment.

We found early in the series of experiments, that the denervated vessels of "one day" rabbits responded best. That is, their response was most nearly a passive one. If a longer time elapsed the denervated vessels showed an increasing tendency, with the increase in the length of the interval, to constrict and a decreasing tendency to react passively to pressure changes within. The following figures will serve to make this point clear.

In 11 experiments we used "one day" rabbits. The denervated vessels invariably showed good passive responses to pressure changes. In 5 experiments we used "three day" rabbits. In four of these the passive response of the denervated vessels was good, but in the fifth scarcely any passive response could be obtained. Two "four day" rabbits were used. One of them failed to show a passive response. Of the two "six day" rabbits used one failed to respond passively. Finally, one experiment each was performed upon "seven day," "nine day," and "twelve day" rabbits respectively. The "seven day" rabbit gave a good passive response but the remaining two showed practically no passive reaction whatever. To summarize these results we may say that in every case when used within one day after denervation (11 cases), the passive reaction of the vessels was good; that they failed to react well in one out of five "three day" rabbits, while in seven experiments upon rabbits of "four days" or over, the failure to respond passively was seen in four, that is, in about 57 per cent.

What is the significance of these results? It is this. We require, for our demonstration, vessels which respond passively to pressure changes within them in order that, by comparison, we may determine the state of tonus being maintained by the vaso-motor center in the vessels with which it is still connected. The results stated above show that to obtain this passive response in the control vessels, "one day" rabbits are best; that, while it is sometimes possible to obtain a passive response, even as late as seven days after denervation, still in many cases the vessels have developed by that time too much autonomy to serve as passive controls.

The above observation agrees with statements in the literature[3] to the effect that the dilatation of the vessels of the denervated ear gradually decreases and finally may even disappear. The time necessary for the latter to develop is stated by Meltzer, however, to be a matter of weeks.

It is stated by Bayliss[4] that the arterial wall reacts to an increase in arterial pressure by constricting. If this statement be true, can we use the denervated ear vessels as passive controls? Anrep,[5] working later upon the same subject in Bayliss' laboratory, concluded that "a local form of reaction of the blood vessel wall to changes of tension is as yet unproven." Even though Bayliss' original interpretation were correct, namely, that if the pressure within an artery is increased, the artery responds by contracting, or, that if the pressure falls the arterial wall relaxes, his results would not necessarily be in conflict with our findings. This is true for the following reasons:

Bayliss does not say to what degree the arteries became constricted and there is certainly no evidence that they responded to the increased pressure with a strong constriction. It may indeed be true that the arteries and arterioles in

[3]Meltzer and Meltzer: Am. Jour. Physiol., 1903, ix, pp. 62 and 159.
[4]Jour. Physiol., 1902, 28, 220.
[5]Jour. Physiol., 1912, 45, 318.

the denervated ears of our animals were not as much dilated as they would have been had they not possessed any such reaction to internal pressure. And especially may this be true of the denervated vessels a number of days after the denervation when they show a definitely greater tendency to remain constricted. That these vessels were more dilated in our experiments after raising the blood pressure than they were before, however, cannot be questioned. In many cases the dilatation was easily visible across the entire width of the room and seemed to be practically maximal.

Again, the constriction described by Bayliss was apparently of short duration. He does not give, in all cases, the full tracing, but in one that he does give (Fig. 7, p. 226) the duration of the constriction was about one minute. In some other cases it was apparently somewhat longer. In our experiments the high blood pressure was usually maintained for from 3 to 5 minutes and sometimes (see protocols below) even longer. A transient tendency to constrict would, therefore, have disappeared in all probability before we removed the aortic clamp. Moreover, in our experiments, the increase in blood pressure developed slowly and not suddenly as was the case in Bayliss' experiments, for the heart does not build up a high blood pressure in the head region instantly upon clamping the aorta. Since usually the efficiency of a stimulating agent varies directly with the suddenness of its application, perhaps the tendency of the vessels to react when the increase in internal pressure is developed slowly is less than when it is rapid.

When all these factors are considered it can be seen that there is not necessarily a conflict between the results obtained by Bayliss and those here described. Perhaps the most conclusive evidence that the denervated vessels do dilate when subjected to a high blood pressure is furnished by the well-known fact that after cutting the cervical sympathetic nerve in the rabbit (as in the Claude Bernard experiment) the ear vessels on the corresponding side become widely dilated. This result obtains in an otherwise normal animal and therefore in one with a high blood pressure. It must be admitted, therefore, that at least to the extent to which these vessels dilate under high pressure and empty themselves when the pressure falls they may be used as passive controls for the blood vessels still connected with the center in the medulla.

Results of the Shock Experiments.

There was a remarkable constancy in the behavior of the vessels of the normally innervated ear. The following protocols are typical of the results obtained:

PROTOCOL I.

Experiment 14. May 15, 1914. One Day Rabbit. White Male. 1700 grm. Yesterday at 4:00 P. M. denervated the right ear.

3:00 P. M. Began operation. Before operation vessels of normal ear well constricted; those of operated ear fairly well dilated. After tying down the animal, vessels of both ears dilated.

3:56 Operation completed. It consisted of tracheotomy, wide opening of abdomen, removal of adrenals and freeing of aorta just below diaphragm. Canula inserted in left axillary artery for blood pressure record.

4:00 Blood pressure 32 mm. Hg. Lid reflex fair. Denervated ear moderately dilated. Normal ear vessels slightly smaller.

4:09½	Clamped aorta. Blood pressure began rising rapidly.
4:11	Vessels denervated ear widely dilated. Normal ear moderately constricted.
4:13	Blood pressure 94 mm. Hg. Lid reflex active.
4:14	Clamp off aorta.
4:15	Denervated ear still moderately dilated. Normal ear pale; vessels strongly constricted. Blood pressure 30 mm. Hg.
4:25	Aorta clamped again. Blood pressure rapidly rose to 87 mm. Hg.
4:26	Denervated ear widely dilated. Normal ear blanched; bloodless.
4:35-40	Cut the auricularis magnus and removed superior cervical ganglion on left side —that is for normal ear. "Normal" ear vessels became maximally dilated. Aorta is still clamped and blood pressure high.
4:45	Clamp removed from aorta. Blood pressure fell at once to 22 mm. Hg.
4:47	Vessels both ears same size (moderately dilated).
4:48	No lid reflex. Blood pressure 22 mm. Hg.
4:49	Clamped aorta.
4:52	Blood pressure 88 mm. Hg. Slight lid reflex. Vessels both ears practically maximally dilated. Clamp off aorta.
4:57	Blood pressure 36 mm. Hg. Slight lid reflex. Vessels of both ears moderately dilated.
4:57½	Clamped aorta again.
4:59	Blood pressure 100 mm. Hg. Vessels both ears maximally dilated.
5:00	Clamp off aorta.
5:08	Animal killed.

In this experiment the blood pressure had fallen within one hour after the beginning of the operation to 32 mm. Hg. pressure—that is to about one-third of the pressure normally present. At this pressure there was only a slight, though definite difference between the vessels of the two ears. When the aorta was clamped at 4:13 and the blood pressure in the head region rose quickly to 94 mm. Hg. a marked contrast appeared between the vessels of the two ears. The denervated vessels became widely distended with blood while the normally innervated vessels continued to be moderately constricted. When the aorta was unclamped the blood pressure fell quickly to a low level again—namely 30 mm. Hg. There was still a difference, though not a marked one, between the ears. At 4:25 the aorta was clamped and the blood pressure rose to 87 mm. Hg. This time the difference between the two ears was even more marked than after the previous clamping, for the protocol reads that this time the normal ear was "blanched and bloodless," whereas before it was described as being "moderately constricted." In other words, it might be thought that the vaso-motor center had not only not lost any of its grip upon the blood vessels during the past fifteen minutes, but had actually increased its tonus. At this point in the experiment the final step in the test was made to determine whether the vessels of the normal ear were constricted because of the activity of the vaso-motor center, i. e., the vaso-motor nerves of the normal ear were quickly exposed and severed. The result was unmistakable, for the "normal" vessels quickly became maximally dilated. At no time thereafter were they again smaller than those that had been denervated the day before. Twice, later, the aorta was clamped and unclamped. The blood pressure rose from 22 to 88 mm. Hg. and from 36 to 100 mm. Hg., respectively. Each time the vessels of both ears dilated strongly with the rise in blood pressure and emptied somewhat when the fall came. That is, they both reacted in the *same* direction, in strong contrast to their reaction before the "normal" ear was denervated.

The following is an example of a one day (20 hours) rabbit in which, to-

ward the end of the experiment, the cervical sympathetic was frozen instead of cut, as in the former experiment:

PROTOCOL II.

Experiment 32. July 30, 1914. White Female Rabbit. 1550 grm. Vessels of right ear denervated 20 hours before shock experiment.

8:10 A. M. Before disturbing animal in cage the vessels of normal ear were strongly constricted, while the denervated ear vessels were widely dilated.

8:15 Began operation. Method exactly same as in Experiment 14 (Protocol I), except that this time adrenals were not removed.

8:50 Operation completed. Vessels of both ears same size; moderately dilated.

9:06 Have been handling abdominal viscera for over ten minutes. Normal ear well constricted; denervated ear well dilated. A marked difference between them. Lid reflex good. Respiration 132.

9:43 Blood pressure 46 mm. Hg. Normal ear well constricted. Denervated ear moderately dilated.

9:44 Clamped aorta.

9:46 Blood pressure 72 mm. Hg. Normal ear still well constricted. Denervated ear vessels gorged with blood.

10:15 Clamp off aorta.

10:25 Again clamped aorta.

10:28 Normal ear vessels well constricted. Denervated vessels widely dilated.

10:30 Froze left cervical sympathetic nerve with ethyl chloride spray. Vessels of "normal" ear became quickly as widely dilated as those of denervated ear.

10:45 Clamp off aorta.

11:08 Blood pressure 30 mm. Hg. Respiration 44 per minute. Lid reflex sluggish. "Normal" vessels slightly dilated. Denervated vessels moderately dilated (i. e., somewhat wider than "normal" vessels).

11:09 Clamped aorta again. Blood pressure rose to 54 mm. Hg. Denervated ear vessels gorged. "Normal" ear (nerve frozen at 10:30) well dilated but definitely less so than denervated ear vessels.

11:10½ Clamp off aorta. Blood pressure fell at once to 23 mm. Hg. Lid reflex sluggish. Animal seems deep in shock. Allowed to die.

The results of this experiment are essentially the same as those of the previous one. The normal vessels remained constricted until the conductivity of the cervical sympathetic nerve on that side was temporarily abolished by freezing it with the ethyl chloride spray. They became then as widely dilated as the denervated vessels. The freezing was discontinued as soon as the vessels of the corresponding ear dilated. That the conductivity of the nerve had not been permanently abolished by the freezing is shown by the fact that 39 minutes later (11:09) when the aorta was again clamped the normal vessels did not dilate so strongly as the denervated vessels. Some permanent injury had evidently been done, however, for the normal vessels did not return again to a state of strong constriction. It will be noted that a good dilatation was obtained in this case by destroying the conductivity of only one of the nerves supplying the vessels of the normal ear.

In the following experiment the control ear was denervated three days before the shock experiment.

PROTOCOL III.

Experiment 34. Sept. 12, 1914. White Male Rabbit. 1250 grm. Left ear denervated three days ago.

8:10 A. M. Vessels of normal ear constricted. Those of denervated ear well dilated.

8:20 Operation began—tracheotomy, abdomen opened wide, aorta exposed just below diaphragm, canula in left axillary artery for blood pressure. Adrenals left intact.

9:33	Operation completed. Blood pressure 80 mm. Hg.
9:40	Lid reflex sluggish. Respiration 48, slow, efficient, regular. Normal ear vessels strongly constricted. Denervated vessels slightly dilated.
9:50	Clamped aorta.
9:54	Normal ear as before strongly constricted. Denervated vessels well dilated. A marked difference between them.
9:57	Clamp off aorta.
10:32	Blood pressure 53 mm. Hg.
10:32½	Clamped aorta. Blood pressure rose rapidly to 108 mm. Hg. Normal ear vessels bloodless as before. Denervated vessels gorged with blood. The difference can be seen across the room without difficulty.
10:37	Clamp removed from aorta. Blood pressure fell to 49 mm. Hg. Heart became irregular and slow. Lid reflex gone. Struggling, apparently dying. Clamped aorta again. Blood pressure rapidly rose to 119 mm. Hg.
10:42	Blood pressure 120 mm. Hg. Normal ear absolutely bloodless and blanched. Denervated vessels gorged with blood.
10:50	Clamp off aorta. Blood pressure fell to 48 mm. Hg.
10:52	Blood pressure very low. Lid reflex sluggish. Respiration 40 per minute, regular, efficient. Normal ear vessels blanched. Denervated ear pink, but vessels slightly constricted. No response to handling of viscera or stroking of parietal peritoneum. Aorta clamped.
10:52½	Ether applied to trunk of right sympathetic.
10:53½	"Normal" ear vessels widely dilated. Somewhat wider, in fact, than vessels of ear denervated three days ago.
10:55	Ether washed off nerve. Clamp off aorta.
11:05	Denervated vessels slightly dilated. "Normal" vessels somewhat smaller than control vessels. Aorta clamped.
11:08	"Normal" ear vessels definitely smaller than those of control ear, which are well dilated. Again applied ether to cervical sympathetic and normal vessels dilated as before.
	Experiment discontinued.

The first point to be noted in this experiment is that the control vessels reacted very satisfactorily to internal pressure changes. If they resisted at all the tendency toward dilatation when the blood pressure was raised by clamping the aorta, it was certainly not to any marked degree, for the word "gorged" described best the picture presented at that time. This engorgement did not confine itself to the main trunk vessels of the control ear. The whole ear was suffused to a marked extent. Inasmuch as three days elapsed between the denervation of this ear and the execution of the shock experiment, we believe it can hardly be claimed that this dilatation was in any sense due to a persistent stimulation of vaso-dilator nerve fibers that were injured three days before when the nerves were cut. The evidence in favor of this view is even more conclusive in the protocol which follows, for in that case *seven* days elapsed after cutting the nerves of the control ear before the shock experiment was carried out, yet the conduct of the vessels of the control ear was essentially the same as in the experiment under discussion.

The conduct of the vessels of the normal ear in this experiment is striking. Two and a half hours after the beginning of the experiment the blood pressure had fallen to 48-49 mm. Hg. (see Protocol at 10:37 and 10:50). When the aorta was clamped at 10:42 the blood pressure rose to 120 mm. Hg.—that is, to a high level for the normal rabbit. The general condition of the animal, at this stage of the experiment, was bad. The lid reflex was sluggish and sensory responses very poor or entirely absent. In spite of the state of the animal, generally, and in spite of the very high blood pressure, the normal ear

(10:42) remained "blanched and bloodless." Furthermore, the vessels remained persistently in a state of what seemed to be maximal constriction until the conductivity of the cervical sympathetic nerve on that side was abolished by the application of ether. Immediately thereafter the vaso-constriction of the normal ear was replaced by a marked dilatation, thus furnishing evidence that the preceding constriction was due to the active influence of the vaso-constrictor center. We wish to call attention to the following additional evidence that we are dealing here with an active vaso-motor center. The ether was washed off the cervical sympathetic as quickly as possible after the vessels of the "normal" ear dilated. This was done to prevent, if possible, a permanent abolition of the nerve conductivity. We had the satisfaction of seeing later a partial recovery of tonus by the vessels of the "normal" ear—that is, these vessels again became distinctly smaller than those of the control ear under the same pressure. That they did not again become strongly constricted, was due in all probability to some permanent injury produced by the ether. A second application of ether to the cervical sympathetic again brought about a strong dilatation of the "normal" vessels.

The fact that the vessels of the *normal* ear became slightly more dilated, after application of the ether to the cervical sympathetic, than those of the control (denervated) ear, illustrates the statement made above that vessels several days after denervation usually show a greater tendency to resist the dilating influence of internal pressure than do vessels that have just been denervated.

PROTOCOL IV.

Experiment 17. June 16, 1914. Seven Day Rabbit. White Male. 1850 grm. Right ear denervated seven days before shock experiment.

8:14 A. M.	Vessels of denervated ear definitely, but only slightly, wider than those of normal ear.
8:15	Began operation. Tracheotomy. Abdomen opened wide, adrenals removed; canula in abdominal aorta at level of bifurcation into iliacs, for blood pressure tracing.
9:08	Began registering blood pressure. Blood pressure 42 mm. Hg. Normal ear vessels wide. Denervated ear vessels small.
9:25	Normal ear blanched. Denervated ear moderately constricted, but vessels definitely wider than normal ear vessels.
9:33	Clamped abdominal aorta above celiac. Normal ear remained blanched and bloodless. Denervated ear vessels fairly widely dilated and the whole ear bright pink in color. Could not register blood pressure while aorta was clamped.
9:38	Clamp off aorta.
9:39	Animal in very deep shock.
9:43	Blood pressure 30 mm. Hg. Lid reflex very sluggish. Normal ear bloodless. Denervated ear well suffused. Vessels moderately dilated.
9:47	Abdominal aorta clamped above celiac again.
9:49	Normal ear blanched as before. Denervated ear vessels almost maximally dilated. The difference between the ears is very striking.
9:54	Clamp off aorta.
10:05	Blood pressure 24 mm. Hg. Lid reflex very sluggish. Respiration regular, shallow, 52 per minute. Normal ear bloodless as before. Denervated ear moderately dilated and suffused.
10:10	Removed left superior cervical ganglion and clamped aorta above celiac.
10:12	Normal ear vessels (just denervated) almost maximally dilated. Wider than the other ear. Denervated ear vessels (former denervation) moderately well dilated.

10:28 Ears same as 10:12. Respiration 24 per minute. Lid reflex sluggish. Heart feeble and slow.
10:30 Clamp off aorta. Struggling. Blood pressure 15 mm. Hg.
10:34 Animal nearly dead. Blood pressure falling gradually. Killed.

As regards the conduct of the vessels of the normal ear the experiment differed in no essential respect from the experiments already described. Before, during and immediately after the operation, these vessels showed evidences of the usual varying intensity of vaso-constrictor tonus (8:14—moderate constriction; 9.08—wide dilatation; 9:25—blanched); later in the experiment, when the blood pressure became very low, they showed no relaxation at any time. Whether the aorta was clamped or not, the normal ear vessels remained practically maximally constricted.

This contrast between the vaso-motor reactions obtained in the early and in the late stages of the experiment was seen so frequently that we may say it was practically a constant phenomenon. The vessels of the normal ear were usually in a state of moderate constriction before the animal was disturbed in the cage and practically always became widely dilated in consequence of the handling, tying down and operating—in short, before the experiment began and during its early stages they were subject to varying degrees of contraction and dilatation. In the late stages, however, there was almost never a variation from a state of vaso-constrictor tonus sufficient to keep the normally innervated vessels strongly contracted. This may be interpreted to mean that early in the experiment, when the general blood pressure control by the vaso-motor center is ample, either a vaso-constrictor or a vaso-dilator response may be made by the center to a change in the environment; that later in the experiment, however, when the blood pressure has shown a strong tendency to fall, the vaso-constrictor center marshals all its forces and only one type of response is seen —namely a strong constriction; that, moreover, this constrictor influence is maintained through a period when other functions show marked signs of giving way.

Again, in this experiment, we see that the "normal" vessels became strongly dilated as soon as the cervical sympathetic path was interfered with. The chief reason for presenting this protocol is that the control vessels, seven days after denervation, reacted in the same sense as did those of the so-called "one day" rabbits. It is surely reasonable to conclude that the dilatation seen in this case in the denervated ear, after clamping the aorta, was due to mechanical distention of the walls caused by the pressure of the fluid within, and not to any vaso-dilator influence of nervous origin. We regard this as a point of vital importance. If it be proven that the denervated vessels respond to increased pressure by a distention, then the normal vessels should, if left to themselves, react in the same way. If they react in an opposite sense it must be due to some outside influence. We believe we have shown by the experiments here described that there is an outside influence and that it can be removed by severing the connection between the vaso-constrictor center and the vessels. Moreover, we believe the foregoing evidence warrants us in concluding that the vaso-constrictor center is not the first of the vital centers to fail. In fact, there is some warrant for believing that at a time when other functions have yielded definitely, the vaso-constrictor center still shows marked activity.

Again in this protocol we find an illustration of a statement made in the

early part of the paper, namely—that if several days elapse after denervation, the denervated vessels show a considerable tendency to establish autonomy. This is evident throughout the experiment.

There is one other fact to be noted in this protocol which was noted also in a number of other animals experimented upon several days after denervation of the ear. The denervated vessels showed a greater tendency to contract at the beginning of the experiment than during the latter part. We do not wish to enter at this time upon an exhaustive discussion of the possible reasons for this apparent paradox. It might be said in line with Bayliss' experiments, that in the beginning the stronger tendency to constrict was the impulse furnished the muscular wall of the blood vessel by the high blood pressure;

Fig. 2.—The rabbit is in shock. The left ear (constricted vessels) is in connection with vaso-constrictor center. The right ear (dilated vessels) has been cut off from central control. (See Protocol V.)

and that as the blood pressure fell, during the course of development of shock, this tendency also diminished and the vessels dilated. Against this view stands the fact observed in several instances that the vessels dilated late in the experiment more than in the beginning, even if the blood pressure, by clamping the aorta, was raised to from 70 to 90 per cent of the normal level. If the vessels were stimulated to constriction by the high blood pressure in the beginning, they ought to respond to pressure in the same way later on. Since we plan to investigate further the pressure reactions of blood vessels, we will leave a further discussion of this point until a future time.

The protocols already presented are representative of the results obtained

in by far the majority of the experiments. We may designate as the type experiment, one in which the development of shock was a gradually progressive phenomenon that could be represented roughly by a straight line with one end placed lower than the other. In some cases the descent into a condition of shock was relatively rapid; in others very slow. The former of these could be represented by a line indicating a steep slope; the latter by a longer line, having a much more gradual slope downward. In both of these the vaso-motor center was strongly resistant. So far as one could judge from the vessels of the normal ear there was no time during the experiment, unless it were at the very end, i. e., just before death, that the vaso-constrictor center was not active (we are not speaking of degree of activity now).

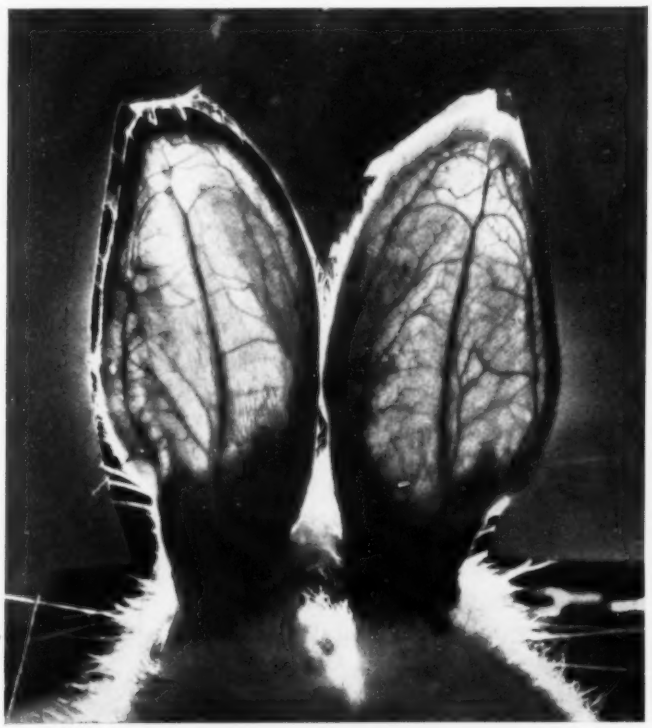

Fig. 3.—Same rabbit as shown in Fig. 2. Photograph taken later in the experiment after the vessels of the left ear had also been cut off from central control. The formerly well contracted vessels of the left ear are now well dilated. (See Protocol V.)

We have three experiments which present a variation from the above type and which throw an interesting light upon this struggle of the vaso-motor center for control of a desperate situation. We may describe this type as one in which the slope, representing the general condition of the animal, is interrupted at one point by a sudden sharp descent of the line, followed by a more or less rapid return of the line to the level of the original slope. Such an experiment is the following:

PROTOCOL V.

Experiment 28. July 15, 1914. Male White Rabbit. 1550 grm. Shock experiment started 24 hours after denervating vessels of right ear.

8:05 A. M. Vessels of denervated ear well dilated. Those of normal ear well constricted.

8:10	Operation began. Method exactly same as in Experiment 34 (Protocol III).
8:50	Operation finished. Lid reflex good. Respiration regular and efficient.
9:03	Blood pressure 34 mm. Hg. Vessels of denervated ear well dilated. Normal vessels well constricted.
9:15	Considerable blood lost accidentally from axillary artery. Struggling. Heart and respiration stopped. Lid reflex gone. Clamped aorta above celiac. Started artificial respiration and cardiac massage.
9:19	Recovering well now.
9:25	Just before animal nearly died, at 9:15, the normal ear was practically blanched, while the denervated vessels were well dilated. Then at 9:19, with the recovery of the animal, the vessels of the normal ear became widely dilated (no vaso-constrictor tonus evident at all). Now the normal vessels are already beginning to constrict. They are definitely smaller than the denervated vessels again.
9:32	Vessels of denervated ear well dilated. Normal vessels practically blanched. Aorta still clamped. Blood pressure 92 mm. Hg.
9:40	Took photographs of ears as at 9:32 (Fig. 2). While taking the photograph, observed that the vessels of normal ear showed excellent rhythmic contractions and dilatations at the rate of 6 per minute. The vessels oscillated between "blanched" and "slightly constricted." The rhythmic changes easily seen at a distance of three feet. Denervated vessels never showed rhythmic contractions.
10:10	Must leave clamp on aorta, otherwise blood pressure quickly falls to near zero and heart stops. Cut left cervical sympathetic and removed ganglion.
10:20	Vessels of left (normal) ear already wide. More photographs (Fig. 3).
10:32	Blood pressure 96 mm. Hg. with aorta clamped.
10:37	Clamp off aorta.
10:38	Blood pressure 12 mm. Hg. Heart stops if clamp is removed from aorta for any length of time. Lid reflex nearly gone.
10:38½	Heart nearly stopped. Clamp on aorta again.
10:45	Lid reflex good. Respiration very slow, regular, and apparently efficient. Blood pressure has risen to 35 mm. Hg. Vessels of left ("normal") ear (denervated at 10:10) slightly wider even than "denervated" vessels. Heart stops quickly whenever clamp is removed from aorta. Experiment discontinued.

Here we see an animal in which (until 9:15) the course of the descent into a state of shock was in no essential particular different from the usual one, which has already been described. At 9:15 an accidental hemorrhage nearly resulted in the death of the animal. Both the heart and respiration stopped and the lid reflex disappeared. Within four minutes, however, through the use of artificial respiration and cardiac massage, the animal had revived and was doing fairly well. The blood vessels of the normal ear, which before the hemorrhage were strongly constricted, showed now a marked contrast to that condition. They were widely dilated, which means that there was little if any evidence of vaso-constrictor tonus. From this time on, however, the vessels gradually became smaller and smaller. At the end of six minutes they were already definitely smaller than those of the control (denervated) ear. At the end of thirteen minutes they were again as strongly constricted as before the hemorrhage. One of the photographs taken shortly after this (Fig. 2) shows something of the contrast between the vessels of the two ears, though it is by no means as striking as the actual picture presented by the ears themselves. The reason for this is twofold. First, the smaller vessels of the denervated ear that were visible to the naked eye do not show up distinctly and the opacity of the larger vessels along their margins seems to have been insufficient to cast a distinct shadow upon the photographic plate. As a result these (larger) ves-

sels seem narrower in the photograph than they actually appeared to the eye. Second, the photograph fails entirely to show the color differences of the two ears. The engorgement of the denervated ear was not confined to the larger vessels, but seemed to involve also those that were invisible to the eye for the whole ground substance of this ear had a definite and marked pinkish flush. The normal ear, in contrast to this, had a cadaveric whiteness which was tinged with a suggestion of yellow rather than of pink. In addition to this blanching of the ear substance, the smaller vessels were invisible and the larger ones reduced to mere threads.

In order to convey a more accurate impression of this contrast, we had the artist make a water color sketch from life (see Fig. 1). While it is of necessity partially schematic so far as the vessels of the ears are concerned, still it gives a much more exact general impression than does the photograph.

Both the photograph (Fig. 2) and the colored sketch (Fig. 1) represent the appearance of the ears at a time when the general blood pressure was so low that the heart stopped when the clamp was removed from the aorta, thus allowing the splanchnic area and the posterior part of the body to be included in the circulation. With the aorta clamped just below the diaphragm, however, the heart built up a pressure in the head region equal to 92 mm. of mercury, and in the face of this pressure the vessels of the normal ear remained constricted as shown in the two figures just mentioned.

A little later in the experiment the vessels of the normal ear were also denervated, whereupon the constriction of these vessels was quickly replaced by a wide dilatation. Fig. 3 is one of the photographs taken at this stage of the experiment. While it fails to bring out the flushing of the ear substance that developed, it does show fairly well the enlarged blood vessels.

The description of the contrast between the normal and the denervated vessels just given holds in a general way for all the experiments in the series. The individual variations that were seen were variations in the degree of the contrast. When "one day" rabbits were used, and the aorta clamped temporarily late in the experiment, the above described differences obtained in by far the majority of the experiments.

As already stated, Protocol V is one of a group of three experiments in which there was evidence of early vaso-constrictor failure. Of the two remaining experiments, which belong to this group, one was in all respects similar to Protocol V. That is, there was only a temporary loss of vaso-constrictor activity after stoppage of the heart, and this was followed by a good recovery. In the third experiment, although the animal recovered sufficiently to breathe without artificial respiration, it was not possible at any time thereafter to remove the clamp from the aorta without causing stoppage of the heart. This was apparently due to the fact that the venous return to the heart was insufficient, for upon removing the aortic clamp the arterial pressure quickly fell to a very low level, e. g., from 62 to 14 mm. Hg., and soon thereafter the heart would slow up and stop. Moreover, during the remainder of the experiment the vessels of the normally innervated ear reacted to internal pressure changes in exactly the same way as those of the denervated (control) ear. All of which means that after the temporary stoppage of the heart and respiration early in the experiment the vaso-constrictor center never gave any evidence of recovery.

We have made no systematic study of the effects of hemorrhage or temporary cardiac failure upon the resistance of the vaso-constrictor center. But the results obtained in these three experiments indicate that if such accidents complicate the situation the behavior of the center is markedly different from what it is in those cases in which shock is produced by operative trauma alone.

We appreciate well the fact that in such studies as this it may always be disputed that the condition studied was really that of surgical shock. We wish, however, to call attention to this important point in connection with our experiments. *It is not essential that our animals be reduced to a condition which will be acknowledged by everyone to be identical with deep surgical shock as usually encountered.* We were convinced that our animals sank finally into such a state. It must be recalled, however, that our problem was to determine whether during the *course of development of shock* the loss of vaso-constrictor tonus is the primary cause of all the remaining symptoms, or whether this tonus persists until other symptoms have become marked. Whether in fact, the activity of the vaso-constrictor center may not be well up to normal at a time when the general blood pressure has fallen to a low level and the other symptoms become strongly developed. This being the case, it was only essential that our animals be well on the way toward a condition of deep shock, and it can hardly be disputed that such was the case.

The method used in these experiments to demonstrate the presence or absence of tonus in the vaso-constrictor center could, we believe, be used as a means of demonstrating vaso-constrictor activity in other lines of experimentation just as well as in shock.

Furthermore, since we have (within certain limits) means for raising the blood pressure at will, the *degree* of activity of this center could be determined by raising the blood pressure until the normally innervated vessels begin to yield, i. e., dilate, under the pressure. We might term this the "breaking point." It might even be possible, by making a series of such determinations during the course of a single experiment, to plot a curve that would represent the behavior, as regards degree of activity, of the vaso-constrictor center.

It has been stated[6] that the left ear of the rabbit is better supplied with vaso-motor nerves than is the right. If this be the case, it would be better in experiments such as these to denervate the already poorly innervated right ear and use it as the control. In nearly all our experiments the right ear was used as the control.

Summary and Conclusions.

1. Denervated blood vessels usually show the best passive response to internal pressure changes when used within twenty-four hours after their denervation.

2. Normally innervated ear vessels are strongly constricted while the animal is sinking into a condition of shock and after shock has developed.

3. This strong vascular constriction persists even though the blood pressure be raised well toward the normal level (at a time when the animal shows distinct symptoms of shock).

[6]Meltzer and Meltzer: Am. Jour. Physiol., 1903, ix, 66.

4. This vaso-constriction is due to the activity of the vaso-constrictor center because (a) the control denervated vessels become strongly dilated under the influence of the same pressure and (b) the constriction of the normal vessels themselves disappears at once if their connection with the vaso-constrictor center is destroyed by cutting the nerves, or by abolishing their conductivity with ether or by freezing.

5. Since a fairly high degree of activity of the vaso-constrictor center can be demonstrated even after the blood pressure has fallen to a low level and reflexes are sluggish we believe it is justifiable to conclude that a paralysis or failure of the vaso-motor center is not the *primary* cause of the other symptoms of surgical shock.

THE PHYSIOLOGY OF THE PARATHYROID GLANDS*

By W. F. Koch, Detroit, Mich.

THERE are in the animal organism a number of epithelial bodies occupying definite anatomical positions. Some of these structures which we call glands, possess duct systems which direct the product of metabolism of their epithelia into some body cavity where this product or secretion carries on a definite physiological activity. On the other hand a number of these epithelial bodies are without ducts so that no products of their metabolism can be directly collected. These are the ductless glands and because of the intimate relation of their cells to the lymph and blood capillaries they have been supposed to elaborate substances which are carried by the blood or lymph streams to the various cells of the body. They have been named therefore the glands of internal secretion. The question of their physiological significance has interested the biologist for many years. Yet up to the present day very little light has been thrown upon the mechanism of their activities.

A brief reference to their phylogenesis will show that they are specializations of protoplasm, developed so as to serve better the requirements of the cells of the complex multicellular organism. Naturally with a development of the organism, from the simple single cell to the large structure of many millions of cells, which may be widely separated and therefore placed in different environments, the variously located cells must not only carry on the vital processes of their simple progeniture, but also according to the principle of adaptation they must specialize in those activities which their environment requires for their best service to the organism. Thus the surface cells become protective; certain other cells become highly contractile and accomplish locomotion. Now with this specialization in function of any cells the generality of function as found in the unicellular organism must be to some extent sacrificed. And the specialized cell becomes dependent, perhaps parasitic, upon other cells for a portion of the activities which it no longer finds opportunity to carry out itself; thus the principle of mutual service is developed. And it may be assumed that

*A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

the glands of internal secretion because of their relation to the blood stream, through this medium, supply vitally important substances to the other cells of the body. We find a concrete example in the chemical structure of the thyroid apparatus. In sponges which have no thyroid gland considerable iodin is found in the skeletal structures. In the higher organism, fishes, mammalian, etc., there is a thyroid gland containing considerable iodin and the skeletal structures contain it no longer. It may be assumed, then, that the thyroid gland with its iodin compound does that metabolic work for the skeletal structures which they once performed themselves when they contained this iodin body. Confirmation of this assumption is observed in diseases of the thyroid apparatus where abnormalities in the skeletal tissues result. The physiological relation of adrenalin, found in adrenal glands, to the sympathetic nervous system substantiate further the interdependence of the various tissues and the service the glands of internal secretion may pay those other tissues which work more directly to the adaptation of the organism. Using the above assumption as a guide the writer has endeavored in this work to elucidate the mechanism of activity of the parathyroid glands which up to the present research though given much attention has remained obscure.

Working Basis and Historical Sketch.

The thyroid glands are two oval bodies located ventrally and laterally to the thyroid cartilage and trachea in the neck. In the neighborhood of these larger glands, or imbedded in them, are four small epithelial bodies. These are the parathyroids. They may be grouped in two pairs, the superior pair being located about the superior poles of the thyroid; the inferior pair generally at the inferior poles. They are flattened, round or oval in shape, and in the dog together present a mass no larger than a lentil. Because of their small size and irregular occurrence, they were not discovered until 1880, when Sandstrom called attention to their individuality.

Histologically, their structure differs greatly from that of the thyroid, with which they are so closely associated. They receive their blood supply from branches of the thyroid artery. Their veins enter into those of the thyroid. The glands have a capsule of connective tissue, which, supporting the larger vessels, dips into the glandular substance, imperfectly separating it into lobules. These lobules may take the form of columns of cells, which anastomose; and these columns may be made of one or several rows of cells, sometimes grouped into round follicles. There are three varieties of cells uniformly observed. Two of these types are larger than the thyroid cells having deeply staining nuclei and slightly staining protoplasm with fine boundaries quite distinct. The other type resembles the thyroid cell being low columnar and arranged on a basement membrane which surrounds, follicle-like, a small lumen, that may be filled with a granular or colloidal material. Of the former type, a minority of the cells are large polygonal with deeply-staining nucleus and deeply-staining granular protoplasm, which because of its acidophil nature, resembles some of the hypophysis cells. These several varieties of cells may perhaps represent, by their differences in appearance, various stages in functional activity. Very striking is the richness of the blood supply. This is of the sinusoidal type. The thin endothelium of the blood capillaries is placed in close apposition to practically the whole

surface of each cell from which it is not even separated by fibrous tissue strands. Frequently, glands are found in which the epithelial cords are split by the accumulation of colloidal material and thus an acinus is developed, which resembles closely the follicles that make up the thyroid gland. For this reason the parathyroids have been looked upon for some time as embryonic thyroids.

Whether they have a physiological significance differing from that of the thyroids was disputed for some time, since their supposedly complete removal from the animal body was not always followed by definite physiological changes. This is not surprising for a complete surgical removal of the glands is sometimes impossible. Occasionally one of the glands may be found in relation to the thymus, or in some other part of the mediastinum. We have learned, moreover, through the work of Halsted,[1] that only a minute portion of parathyroid tissue is capable of rapidly growing sufficiently large to carry on the work of all the glands. This was proved by carefully removing all the glands that could be found in a favorable experiment and then transplanting a small piece of a parathyroid in the abdominal wall. It was found that with this small piece of grafted parathyroid tissue the animal behaved quite normally for a long time. After the transplant was removed, however, the typical complex of symptoms developed, which characterizes the parathyroid insufficiency. It is not surprising, therefore, that the older observers (Forsyth) looked upon the parathyroids as incompletely developed accessory thyroids, supporting this view with the observation that after removal of the thyroids, the parathyroids rapidly changed their structure to resemble that of the thyroids. In order to explain the frequent appearance of the syndrome of parathyroid insufficiency, they assumed that such changes were brought about by injury to the superior laryngeal nerve, or other tissues of the neck region (Munk).

The capacity of the parathyroid to take up the thyroid function does not exclude, however, an independent significance; and that these glands mean something more than the thyroids was shown by Beidl, Moussu,[2] Glay,[3] Vassale and Generale,[4] when they pointed out that the removal of the thyroid produces simply a condition of cachexia and the changes associated with myxedema, whereas, removal of the parathyroids is responsible for a typical nervous symptom-complex.

This syndrome, though often referred to as typical, has been rather incompletely described in the literature. Several detailed protocols are, therefore, submitted. The behavior of the parathyroidectomized dog may coincide with either of two distinct types of symptoms, or with a mixture of these types, in which either may predominate. In one type the dominant feature is over-excitability; in the other under-excitability. In the former tonic convulsions are characteristic; in the latter we observe a peculiar muscular flaccidity and a general depression of the nervous system. In either case a pathological condition develops within one or a few days after removal of the glands and proves fatal within two to ten days. The first type is illustrated in the following protocol:

Dog (3) was completely parathyroidectomized December 6, 1913, at 7 P. M. At 8 A. M. December 7th, he had recovered from all visible effects of the anesthetic and operation. He seemed fairly bright and active until 11 A. M. December 8th. At this time the first symptoms of tetany were noticeable in a wrinkling of the forehead and twitching of the right ear. At 12 o'clock the

twitchings were visible in the shoulder muscles and the hind limbs were somewhat extended. During this time the heart rate had increased to 160 per minute; the respiration became more rapid and somewhat labored. At 2 P. M. the dog was found in complete tetany, lying on his side with limbs extended and with opisthotonos; the jaws were locked and the lips raised. The eyes, because of retraction of the lids, seemed to bulge out, the pupil was dilated and the sclera injected. Salivation and lacrimation were profuse. During this convulsion the respirations were extremely difficult, each inspiration and expiration producing a sound suggestive of laryngeal spasm. Inspirations were deep and expirations seemed incomplete and difficult. At about 2:15 the dog attempted to rise to his feet, but his struggles were futile since flexion of the limbs was impossible. By 2:50 the respirations had become shorter and more rapid with the production of less sound, and it was noticed that although the hind legs were extended, one of the fore limbs was now flexed, and instead of being in general tonic spasms, the muscles now played in twitches. A similar twitching was also observed in shoulder and trunk muscles. By 3:30 the respiration changed to a rapid panting; the muscles of the fore limbs were more flaccid, while those of the hind limbs exhibited twitches. The face still retained a peculiar expression with raised lips, wrinkled nose and brow and ears drawn back. He rested in this condition until about 7 P. M. when another and similar convulsion developed which did not subside until after midnight. At 6:30 A. M. the subject was found dead in the cage in an attitude of opisthotonos. Necropsy showed a hyperemia of all of the viscera. The ordinarily invisible intestinal vessels were so dilated as to be easily traceable, the liver and spleen were markedly congested, the bladder filled with urine and the intestinal tract with fluid.

The most striking histological changes occurred in the blood, liver, kidney and brain. The blood of the vena cava and heart of this and all animals showed extensive ante mortem coagulation. White clots in several cases were continuous from within the heart chambers down the vena cava to its iliac bifurcation. They nearly filled the lumen of the vessel. Upon section of the liver, the vessels showed fragmented erythrocytes, many normoblasts, erythroblasts with mitotic nuclei and a small proportion of erythrocytes that stained brilliantly in eosin; the remaining red cells in large areas were blood shadows. Each section of the liver and lung showed a number of large mononuclear cells with eosinophil granules. There were also present a larger number of large flat cells staining very intensely in eosin. These showed no definite granulation. In places they were found to line the smaller veins like endothelial cells. In these places no endothelial cells could be observed. The cells of the hepatic cords showed advanced fatty degeneration of the protoplasm. The nuclei of large areas had disappeared entirely in places where the cell form was fairly well preserved. Such areas were surrounded by circular areas of cells in which the nuclei had become densely stained clumps of chromatin. In the livers of four of the dogs only a diffuse chromatolysis could be observed.

All kidneys showed marked congestion and hemorrhage in the cortex, some anemic, and others, congested medullæ. Some glomerula had lost Bowman's capsule and were hemorrhagic, others were markedly congested. In some of the convoluted tubes the epithelium had degenerated.

The spleen contained a large quantity of pigment. Some of the cells showed chromatolysis.

The lung showed edema, congestion and the blood changes mentioned.

The brain sections, which I prepared in Professor Barrett's laboratory, showed cells in the motor areas with partial loss of Nissl substance and typical tetany nuclei. Various degrees of chromatolysis were also observed in these nuclei.

The intestinal tract besides marked congestion showed in the duodenum and pyloric end of the stomach disintegrating epithelial cells. Their nuclei were converted into solid deeply-staining clumps. These appeared like those in the process of extrusion from the normoblasts.

It is observable that the totality of the symptoms points to a hyper-excitability of the whole nervous system including those neurons contributed by the cord to the sympathetic system. Now the action that may elicit so striking a positive phase, may also be expected to present a negative phase in which a general depression of the central nervous system results. Out of 47 dogs, 2 such cases were found. The following protocol will illustrate:

Dog (31) was operated upon March 8, 1914; at 2 P. M. it recovered from the anesthetic and was apparently normal until March 10th, when instead of walking about the cage and welcoming its attendant, it exhibited no recognition of his presence. It was examined and found lying asleep in a peculiar state of flaccidity with limbs somewhat flexed. When a limb was moved or the head turned back it retained the attitude given it. The subject was not observed to move during the day. It remained in this condition until March 12th when, at 2 P. M. it was found dead in the attitude of dogs that die in tetany. A post-mortem examination revealed no signs of pneumonia or other infection. We, therefore, believe that the dog succumbed to parathyroid insufficiency. This case presents perhaps an over-stimulation of the central nervous system, comparable to shock, a state which may be compared perhaps to the reversing of a chemical reaction by the products of the reaction.

The regular occurrence, after complete parathyroidectomy, of a typical symptom-complex facilitates the study of the mechanism by which these glands functionate, since a study of the causation of the tetany should reveal the position of these glands in the metabolism of the organism. Previous to this research, only one contributing fact has been brought forth; it is the discovery by MacCallum[5] that the urines of parathyroidectomized animals contain excessive quantities of calcium; and that when calcium salts are injected intravenously into such animals, the tetany is immediately controlled. MacCallum[6] expressed the view therefore, that the parathyroids had to do with the metabolism of salts but more especially the calcium salts; and he referred to this substance a special physiological value, such that when it was lost from the animal body a calcium deficiency resulted that constitutes the essential pathological condition of parathyroid insufficiency. It was shown by Beebe[7] and Beebe and Berkeley[8] that injections of other salts have similar though not so marked an effect. These observers showed that the length of time over which aqueous solutions of calcium or other salts are useful in controlling the tetany, is relatively short, varying between one and several days. It appears that if calcium insufficiency were the essential change the addition of calcium to the body through intra-

venous injection should alleviate the pathological condition so long as this treatment was used.

In a recent investigation the writer found that when the tetany became uncontrollable through injections of aqueous salt solutions the kidneys had become so pathological as to be unable to functionate normally. Since one of the effects of such intravenous injections is diuretic, aiding the elimination of toxic substances from the blood, it may be assumed that one of the beneficial effects of the aqueous calcium injections depends upon increasing the work of the kidneys and thus the detoxication of the blood. That calcium should herein be more valuable than other salts may depend upon its depressing qualities, but the fact that it is a diacid base would indicate a value as an acid carrier greater than that possessed by the monovalent metals. Therefore when excessively excreted from the body a large number of acid radicals are lost and when injected a large content of acid radicals is added to the blood. If then the value of calcium depends upon the increasing or maintaining of a certain reaction of the blood, the acid radicals are here the important factors. They present two possible modes of activity, the simple neutralization of basic substances excessively elaborated, within the body or the destruction of such substances as are capable of producing the tetany.

There is still another source of indications that aid in the directing of the present investigation. We expect that when a vital process is removed from the organism, the dependent processes will come to a standstill. If this be true some hitherto useful substance should be present and excreted from the organism unused as fast as it is offered for metabolism. Such a substance must contain vitally reactive groups, and if these groups are not normally taken care of they present the possibility of disturbing other vitally reactive substances in the organism and thus of becoming toxic.

There are then several indications that the tetany of parathyroid insuffi-

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RECORD A-I. (See opposite page.)

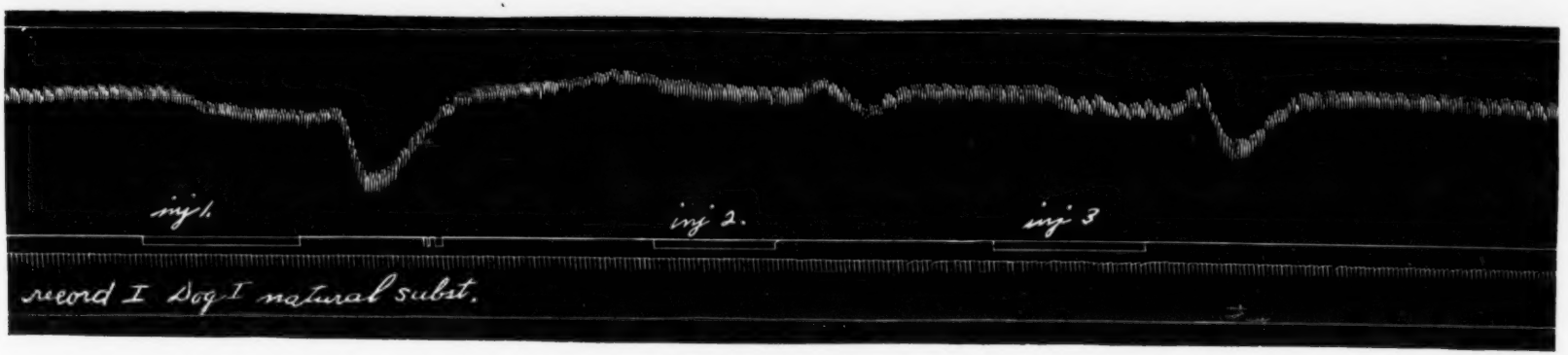
(Dog 1)

Injections given by jugular vein.
Quantity—5 c.c. 0.6 mgms. of natural substance.
Fall in pressure from first injection.....about 30 mm. Hg.
Fall in pressure from second injection.....about 5 mm. Hg.
Fall in pressure from third injection.....about 20 mm. Hg.
Heart rate before injecting.....114 per minute.
Heart rate after injecting.....114 per minute.
Interval between injection 1 and injection 2.....about 72 seconds.
Interval between recovery from first and administration of second injection, about 36 seconds.
Interval between injection 2 and injection 3.....about 45 seconds.

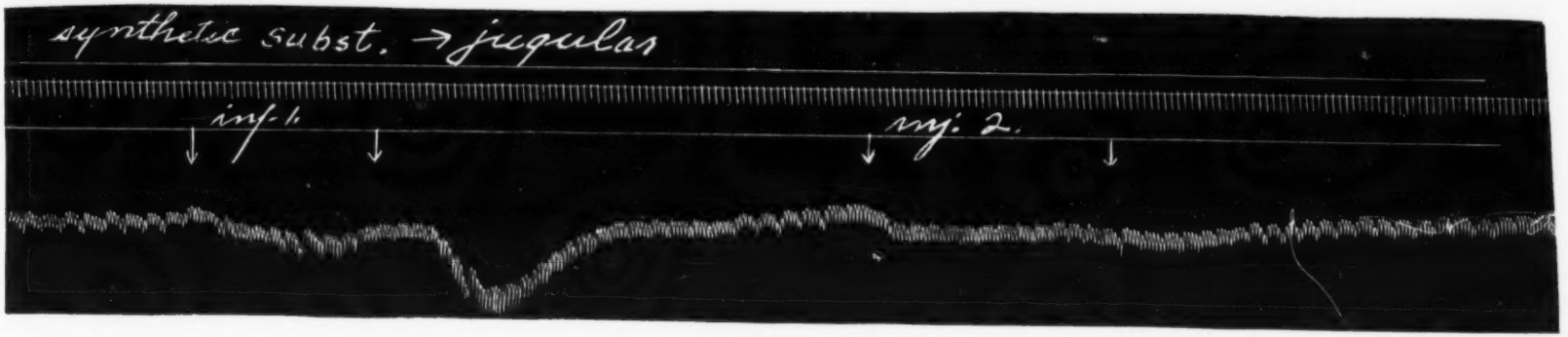
RECORD A-II. (See opposite page.)

(Dog 1)

Injections given by jugular vein.
Quantity—5 c.c. 0.6 mgms. of synthetic substance.
Fall in pressure from first injection.....about 25 mm. Hg.
Fall in pressure from second injection.....0 mm. Hg.
Heart rate before injecting.....138 per minute.
Heart rate after injecting.....136 per minute.
Interval between injection 1 and injection 2.....about 60 seconds.
Interval between recovery of first and administration of second injection..about 30 seconds.



RECORD A-I.



RECORD A-II.

ciency is due to an intoxication. Namely, that it is subdued by increased diuresis and by the neutralization of toxic basicity or the destruction of a toxin by acidity. That the origin of the hypothetical toxic substance is the body itself, that it is useful and not toxic in the presence of the parathyroid glands, that it is filterable through the glomerulus of the kidney (and thus readily diffusible as is shown by the value of diuresis in controlling the tetany) point to a substance hormone-like in nature and therefore very unstable chemically.

It is the object of the present investigation to ascertain the presence and identity of such a substance in the urines of parathyroidectomized dogs and study its physiological properties. As may be anticipated the isolation of an unstable substance from the complex urine presents several difficulties which exclude the hope of quantitative results. My object therefore was to isolate this substance in only sufficient quantities for identification and experimental purposes. After a careful study of the urines the following method was adopted. Its special advantages depend upon its simplicity, rapidity and the avoidance of destructive chemical reagents.

The Method of Isolation.

The urines were collected separately from forty-seven parathyroidectomized dogs. Especially designed cages were used to avoid fecal contamination. The urines were filtered and evaporated to a syrup by an electric fan at a temperature not above 20° C. The residues were dissolved in alcohol, filtered and evaporated, and this process repeated until the last evaporate dissolved readily in alcohol. The lipoids present were extracted with ether and the residue taken up in water. This solution was cautiously precipitated with picrolonic acid. Several insoluble picrolonates were thus obtained, and by recrystallization from water and alcohol were purified.

These substances were tested for physiological activity. Two of them were found to modify the blood pressure when injected intravenously into anesthetized dogs. When injected intraperitoneally into non-anesthetized animals they exhibit very marked toxic effects. They were therefore selected for analysis.

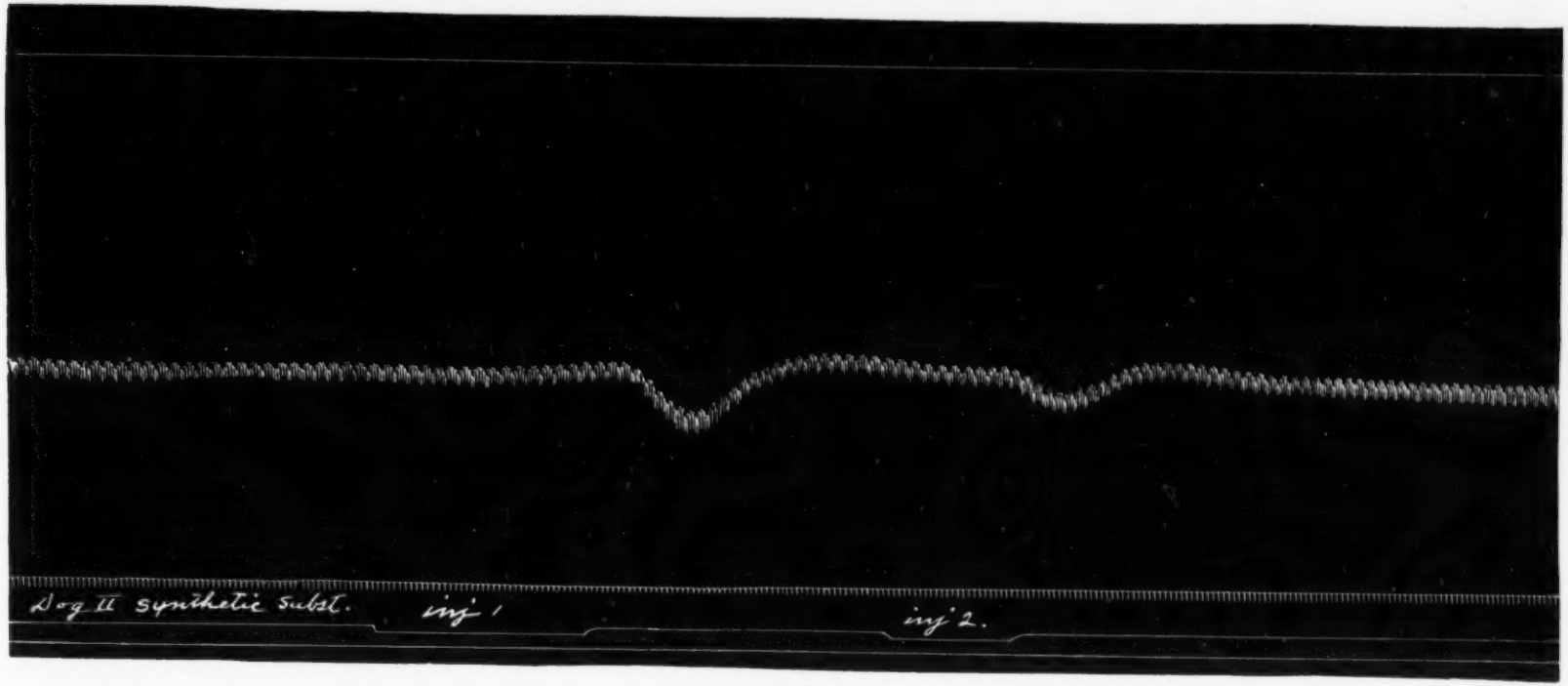
One substance was found to reduce gold chloride quite rapidly, and picrolonic acid slowly. I therefore tested for an aldehyde group. With ammoniacal silver nitrate no mirror was obtained but instead a yellow gray precipitate whose solubility resembles that of silver cyanide. The substance itself freed from picrolonic acid is practically neutral in reaction, very soluble in water and alcohol, somewhat soluble in ether. It gives a picrolonate in the form of very fine

RECORD B. (See opposite page.)

(Dog 2)

Injection given by femoral vein.
Quantity—5 c.c. 0.4 mgms. of synthetic substance per kilo body weight.
Fall in pressure from first injection.....about 25 mm. Hg.
Fall in pressure from second injection.....about 10 mm. Hg.
Interval between injection 1 and injection 2.....about 50 seconds.
Interval between recovery from first injection and administration of second, about 20 seconds.

Note: The heart rate cannot be estimated because while taking the tracing, the recording pointer of the chronograph fell off. After the tracing was finished, we attempted to supply a time record, but it did not prove to be synchronous with the former rate of movement of the drum, which was running down. The time record is therefore valueless.



RECORD B.

microscopic needles which melt at a 118° C., solidifying rapidly to an orange-colored mass which melts with decomposition at about 230° C. 0.249 gms. are soluble in 100 c.c. of hot water.

Upon analysis it gives the following percentage composition.

0.1060 gms. substance gives 26 c.c. N. at 24° C. and 746 mm.
0.1783 gms. give 0.2933 gms. CO_2 and 0.0696 gms. H_2O.

	Found	Calculated for $(C_2H_4N_2)$ $(C_{10}H_8N_4O_5)$
N	26.49	26.31
C	44.86	44.97
H	4.34	3.75

The substance agrees in percentage composition with the picrolonate of methylcyanamide. Methylcyanamide was prepared synthetically from methyl mustard oil. It resembles the naturally occurring substance both in reaction and solubilities. Its picrolonate was prepared and found to melt at 116° C. solidifying immediately to an orange-colored mass which melted at 230° C. with decomposition. In this respect the picrolonate agrees in its behavior with the picrolonate of the naturally occurring substance.

The other substance isolated from the urines and having a physiological action is basic in reaction. It is less soluble in water than the first substance. Its picrolonate crystallizes from water forming small orange-colored mounds. The picrolonate melts at 232° C. with decomposition. It gives on analysis the following percentage composition.

0.1096 gms. give 26.2 c.c. N. at 24° C. and 750.7 mm.
0.1288 gms. give 30.2 c.c. N. at 22.5° C. and 751.3 mm.
0.1731 gms. give 0.2827 gms. CO_2 and 0.063 gms. H_2O.
0.1345 gms. give 0.0520 gms. H_2O and 0.2184 gms. CO_2.

	Found		Calculated for
	A	B	$(C_6H_{12}N_6)_3$ $(C_{10}H_8N_4O_5)_3$
N	26.6	26.28	26.31
C	44.54	44.28	44.97
H	4.07	4.32	3.75

The substance agrees in composition with the picrolonate of trimethylmelamine which is the polymer of methylcyanamide. The polymer was prepared from the synthetic methylcyanamide and found to agree in reaction with

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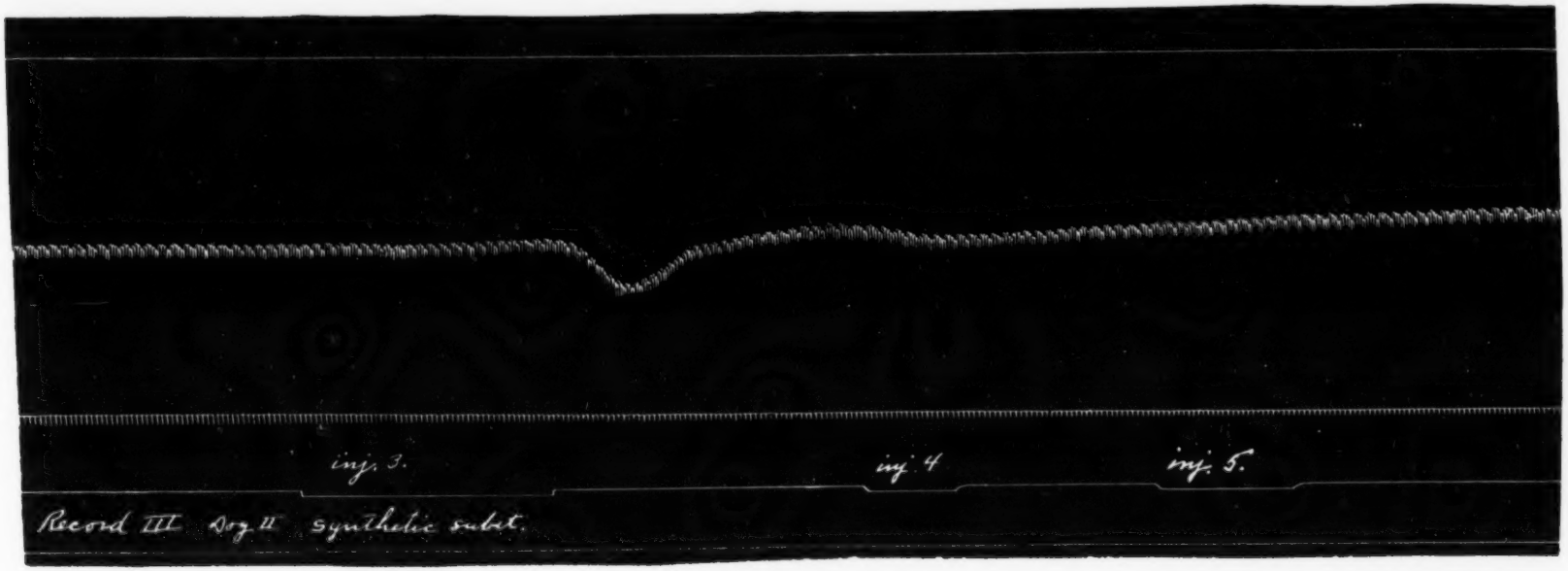
RECORD C. (See opposite page.)
(Dog 2)

Injection given by femoral vein.
Quantity—5 c.c. 0.4 mgms. of synthetic substance per kilo body weight.
Fall in pressure from injection 3.....about 25 mm. Hg.
Fall in pressure from injection 4.....about 0 mm. Hg.
Fall in pressure from injection 5.....about 0 mm. Hg.
Heart rate before injecting.....108 per minute.
Heart rate after injecting.....108 per minute.



RECORD C.

the natural substance. The picrolonate of the synthetic polymer was found to melt at 229.4° C. which is slightly below the melting point of the natural salt. This discrepancy can be explained by the presence of small quantities of methylguanidin in the synthetic preparation which owing to the similarity in solubility of the two picrolonates, could not be completely removed by fractional recrystallization. It was found that methylcyanamide polymerizes after a few days' standing or several evaporations of its watery solution. Therefore the polymer should be expected in the urine from which the cyanamide was isolated.

Because of the agreement in chemical and physiological properties (discussed below) the substances may be considered identified as methylcyanamide and trimethylmelamine.

Physiological Properties.

The methylcyanamide isolated from the urines and the synthetic methylcyanamide were injected intraperitoneally in non-anesthetized dogs and found to have similar effects. In small doses they produce extreme vasodilatation observed in the reddening of the sclera and swelling and reddening of the tongue. Larger doses cause paralysis and convulsions. Still larger doses cause an extremely rapid death. Intraperitoneal injection of 27 mgs. of the synthetic substance into a rabbit of one and one-half kilos' body weight produced after sixteen minutes very marked vosadilatation observable in the air vessels, and a very labored breathing accompanied by wheezing suggestive of bronchial stricture. In this condition the animal rapidly developed tremors and tetany with the head thrown back and hind limbs extended. After a few minutes the tetany gave way to coma resulting in death. An injection of 22 mgs. of natural substance in a rabbit of one and one-quarter kilos produced death very similarly although the tetany was not marked and the dyspnea and coma symptoms predominated. On the other hand 4 mgs. injected intraperitoneally into a white rat of about a 100 gm. weight caused death practically instantaneously with opisthotonos developing no coma whatever.

In order to obtain the base for injection the picrolonate was dissolved in a small quantity of alcohol and decomposed with a calculated quantity of sodium carbonate. The picrolonic acid is thus precipitated as a sodium salt and filtered off. The filtrate was evaporated by an electric fan to dryness and the residue dissolved in water. In this way a solution of known concentration was obtained. The synthetic substance was prepared fresh each time it was used, and from a known quantity of methylthiourea.

Blood pressures were taken by canulæ in the carotid, from dogs anesthetized with chlorotone. For record A-I, the natural substance, for record A-II, the synthetic substance was injected into the jugular vein. For records B and C, the synthetic substance was injected into the femoral vein, experience having shown that injecting into the jugular disturbed the canula.

Record A-I shows the effect of injection of an aqueous solution containing 0.6 mgms. of substance per kilo body weight. Five cubic centimeters of this solution were given with each injection. The first injection was immediately followed by a sharp drop of about 30 mm. of mercury in blood pressure. A second injection of the same quantity given fifteen seconds after recovery from

the first showed only a slight change; a third injection following the second, by the same interval, had an effect less than the first and more than the second. It appears that the animal had become noticeably refractory to the substance after the first injection, and had recovered from this refractoriness considerably at the time of the third. This refractoriness is observed whether the natural or synthetic substance is injected, and its duration toward either substance varies with the animal. In our experience older dogs have a longer refractory period than younger dogs. The dog from which record A was taken recovered almost completely after 140 seconds; the dog from which record B was taken required between 80 and 360 seconds for a complete recovery.

To explain this refractoriness and the quantitative relations discussed below, it might be assumed that the methylcyanamide acts in conjunction with some receptor substance slowly elaborated by the body to produce the vasodilatation and thus the fall in blood pressure. The refractoriness would then be due to exhaustion of the receptor.

In record B the first injection did not use up all of the receptor since an injection following by about 25 seconds, the return of blood pressure to normal, still produced an effect. That detectable receptor is not generated in this dog in 25 seconds is shown in record C where injections 4 and 5 caused practically no pressure change though given at much longer intervals. It appears therefore that injection 4 produced its fall by working with excess of receptor not used by injection 3. The above described relations of the cyanamide and the receptor indicates that they react quantitatively to produce the fall in blood pressure.

It is observable in all of the tracings that any excess of the cyanamide not reacting with the receptor to produce a fall in blood pressure is nevertheless rapidly disposed of, since where sufficient time has elapsed for the generation of noticeable quantities of the receptor, no fall in pressure occurs until a further injection of the cyanamide is given. It may be this reaction of the cyanamide in combining with other groups in the organism that produces the tetany and other symptoms leading to the death of the animal.

It seems that the substance is excreted by the parathyroidectomized animal in considerable quantity. For despite its instability, from the urines of 47 experimental dogs 1.2 gms. of the cyanamide were isolated and 2 gms. of the polymer, as picrolonates. The quantities actually present in the urines or the portions decomposed during the isolation cannot be surmised. Nor would such data be an index to the quantity generated in the organism since during the process of intoxication a considerable portion would be disposed of.

A comparison of the toxicities of the urines of a dog before and after parathyroidectomy shows that during non-fatal tetany the urine is somewhat toxic but that after fatal tetany, the urine is much more toxic. This is shown by the following experiment: A dog weighing 18 kilos. was parathyroidectomized. Eight c.c. of the urine excreted before operation, produced no marked toxic symptoms when injected into a rat of about 100 gms. Five c.c. of 90 c.c. of urine excreted during the half day in which the dog was in moderately severe tetany, upon injection into another rat of the same size produced mild opisthotonos from which the rat recovered in about ten minutes. The urine, after death, in a quantity of 5 c.c. produced severe and almost fatal tetany upon in-

jection into a third rat of equal weight. The urines obtained between tetany periods in quantities of 5 c.c. were not toxic. It may be approximated then that a dog of 18 kilos, and excreting about 200 c.c. of toxic urine gives off from 10 to 80 mgms. of the cyanamide, if we assume that the 5 c.c. injected contain about 1 to 2 mgms. of the substance since 4 mgms. is toxic to a rat of equal size.

The similarity in the behavior of the parathyroidectomized dogs, to that of the non-anesthetized animals treated with the substance isolated from the urine, is further indication that this substance is responsible for the symptom-complex of parathyroid insufficiency. The data therefore justifies the following conclusions:

1. Somewhere in the body methylcyanamide is generated.
2. This substance has a physiological value in normal animals.
3. After parathyroid extirpation the substance accumulates to toxic quantities, and is responsible for the death of these animals.

A further study of some of the problems developed from this investigation is receiving attention.

Operative and Post-Operative Procedures.

The operation consists of complete removal of the thyroid and parathyroid glands, together with the surrounding capsular tissue. The essentials of the operative technic are:

1. Thorough asepsis.
2. Prevention of post-operative hemorrhage.
3. The least possible shock and impairment of vitality.

The aseptic methods are those common to most operating rooms and scarcely require detailed description. The same rigid precautions must be observed as in any major operation. The reason for this care is that the oozing of blood and lymph from the rich capillary bed in which the glands are implanted produces ideal media for the development of pyogenic organisms. This is to be avoided not only because the well-being of the animal is reduced by such infection, but also because it is desirable to obviate as far as possible any discharge from the wound. Another feature not to be ignored in the matter of bacterial growth in the wound is that the absorption of its products may affect the constituents of the urine. A specially devised mask that covered the muzzle of the animal but could not slip down on the field of operation was used. The field of operation was shielded from the mask and hands of the anesthetist by a sterile cover. All the loose dirt and hair are removed from the animal's body by a thorough scrubbing with soap and water and a subsequent drenching with warm 1-1000 bichloride of mercury solution. This is done some time prior to the operation.

To prepare the field of operation, the animal's neck is shaved (over the entire anterior aspect) from the angle of the mandible to the juncture of the neck with the thorax. This is best done immediately prior to the operation. After shaving, the skin is scrubbed with soap and water, dried with alcohol, and then painted with iodine.

Operation.—The following landmarks must be identified: hyoid bone, sterno-

mastoid muscles, larynx, thyroid notch, and trachea. The operator must carefully keep the animal's head directly in line with the rest of the body or the relationship of the parts will be distorted.

Incision is made from a point exactly at the middle of the inferior border of the hyoid bone to a point in the midline at the third ring of the trachea. The first point may be determined by palpating the notch, between the two thyroid cartilages of the larynx, which locates the midline precisely. Such an incision will be about three inches long. The superficial cervical fascia is then incised by gently drawing the knife over its surface. This exposes the platysma fascia which is cut in line with the original incision. It is better to cut through these fascia separately as I have described, because occasionally the anterior jugular vein or some of its larger branches cross the line of incision, and these may be clamped before cutting through the platysma fascia. Thus no hemorrhage occurs to obscure the field or cause unnecessary delay. Passing through the platysma fascia or superficial layer of the deep cervical fascia, the infrahyoid muscles overlapped by the sternomastoid muscles lie in close proximity on either side of the midline. The point of a four-inch scissors is then inserted in the furrow between the inner borders of the two muscles and the intermuscular septum severed by spreading the scissors. This septum is more readily identified if care has been taken to make the previous incisions in the midline and the animal's neck has been retained in the anatomical position. The last step brings us down to the surface of the trachea and larynx covered only by the middle layer of the deep cervical fascia. In the dog, the thyroid gland is represented by two lobes, one on either side of the trachea, each lying in a fibrous sheath derived from the middle layer of the deep cervical fascia. As a rule there is no isthmus or pyramidal process as in the human thyroid. There was a marked variation in size of the individual thyroids we examined as well as in their position, some being immediately below the larynx and others occurring even an inch lower.

One of the lobes is now sought for and brought up into the wound together with the loose fibrous tissue attached to it. It is then seized with a strong hemostatic forceps and light tension is brought to bear on the two poles by which it is fastened. The main branch of the inferior thyroid artery, together with its accompanying vein, is then clamped along with a part of the capsular fascia which is a loose structure and is readily drawn up. The artery is then ligated proximally to the clamp and the ligature anchored in the fascia. A ligature is also passed around a portion of the remaining fascia in the clamp and this is also anchored if it includes many vessels. The artery and portion of fascia thus ligated are then cut between the ligatures and the hemostat. The assistant should hold the stump with tissue forceps as it is cut and not release it until it is ascertained that there is no bleeding. The fascia remaining attached to the lower portion of the gland is caught up in a hemostatic forceps and fractionally ligated, a small portion being included in each ligature. The importance of rigid hemostasis must not be underrated for it is to be borne in mind that following this operation there is a marked vascular dilatation all over the body and considerable oozing may take place from vessels which at the time of operation appear insignificant. If the capsule is carefully ligated in this manner, we are certain to control a great deal of lymphatic seepage. Care

must be taken when clamping the capsular tissue that the descending branch of the ansa hypoglossi is not included. If raised it may be pushed out of the field by gently wiping with dry gauze. The recurrent laryngeal nerve which supplies the intrinsic muscles of the larynx must not be cut as stridulous breathing may occur to say nothing of the discomfort the animal would incur in the loss of his powers of vocalization. It should be identified and pushed out of the field of operation. Occasionally small vessels pass into the gland from the sternothyroid muscle and these must be securely ligated.

After the inferior pole has been freed it is drawn well up out of the wound. This brings the superior thyroid artery with its branches and the superior thyroid vein into view. The artery gives off a number of small branches close to its origin, the most of which are distributed to the glands, others going to the infrahyoid muscles and larynx. The parathyroid bodies frequently lie in the adventitia of this artery and the surrounding tissue. For these reasons it is necessary to ligate the artery close to its origin. Occasionally the artery is very short, so that there is scarcely room to insert a hemostat between its origin from the carotid and the gland. These are factors that increase the necessity for making ligatures very secure. The largest branch is first clamped and ligated, the capsule is then clamped, a small portion at a time and the vessels held in the clamp ligated and cut. The ligatures must be drawn tight, but not so tight as to cut through. The operator proceeds in this manner from the lower outer portion of the capsule about the superior pole of the upper and inner extremity. It is best to secure the larger vessels in a hemostat before cutting. The hemostat is left on the stump until all the vessels have been cut. This can only be practiced where the artery is long enough to permit of a long stump. At the upper and inner part of this pole several small vessels from the trachea will be encountered (the interior tracheal vessels which are branches from the inferior laryngeal). They are sometimes overlooked and produce considerable bleeding. The ligatures are all strongly anchored in the fascia. Before releasing the stump, it must be seized with the tissue forceps and carefully sponged and inspected for any oozing. If care has been observed to include every bit of connective tissues at the superior pole between the ligatures, there will be no bleeding. In this manner the gland is dissected out from below upward and everything is ligated as it is cut. We proceed in the same manner with the gland on the opposite side. After both lobes are removed the region is carefully inspected for accessory lobes. When any are found they are removed.

The wound is closed by three rows of sutures. The sternohyoid muscles are approximated by three interrupted sutures placed equal distances apart. We used a small curved needle without a cutting edge for sewing these muscles. A row of continuous sutures is then put in the platasma fascia. The skin is closed with interrupted sutures. No drainage is used and if the technic has been properly observed there will be little tumefaction and little discharge. The dressing consists of dry gauze harnessed about the neck and shoulders. This is applied during the recovery from the anesthetic.

I shall briefly mention some of the measures used in preventing shock. Kindliness is very essential. In most cases we find that by gaining the confidence of the animal we are able to place it on the board and shave and cleanse

the field of operation before beginning the anesthesia. This alone considerably shortens the time of anesthesia. Ether or ether chloroform and alcohol mixture was used. The anesthetic is begun very gradually. The mask is first placed on the head without the anesthetic, when the animal is quiet and not frightened. The animal is allowed to toss the mask off a few times until it becomes reassured and then a few drops of ether are administered. If force has been avoided up to this point, the animal will breathe the anesthetic without much persuasion. As he feels the influence the rate of administration is gradually increased. In this way, many animals have been carried over to complete anesthesia, practically avoiding the excitement stage.

After recovery from the anesthetic the animal is wrapped in warm blankets which are changed every 5 or 10 minutes. The room is kept warm and the cage scrupulously clean. Because of the tendency towards vomiting after removal of the parathyroid glands solid food is not given. Plenty of fresh water was supplied and this was loaded with lactic acid bacilli with the hopes that their presence in the intestines would prevent the production of toxic amines formed by decarboxylation of amino acids. Animals showing signs of infection or hemorrhage were discarded.

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FURTHER STUDIES IN NICOTINE TOLERANCE*

By Charles W. Edmunds, M.D., and Maurice I. Smith, M.D., Ann Arbor, Mich.

THE question as to how the body acquires the condition of insusceptibility to the effects produced by the introduction of various poisons is one which has always excited a good deal of interest from both the scientific and from the practical standpoints. That such an insusceptibility is developed is well recognized by everyone, but the mechanism by which it is formed is in many cases quite obscure, while in the case of other drugs recent investigations have shed a good deal of light upon the question.

It is unnecessary to enter into a lengthy discussion of the matter in this place as there have been many papers dealing with different phases of the matter, and recently an address by Dixon[1] gave a summary of much of our knowledge upon this subject. In this address, Dixon after discussing the explana-

*From the Pharmacological Laboratory of the University of Michigan.
[1]Dixon: Proc. Royal Soc. of Med., v, 1911, 1.

tions which have been advanced as to the mechanism of the tolerance which is gained by the body to the effects of many of the poisons, dwells at greatest length upon the question of nicotine tolerance. This general consideration of nicotine was supplemented the following year[2] by a detailed account of experimental work which he had carried out in the University of Cambridge in collaboration with W. E. Lee. As a result of the experiments outlined in this paper the writers draw the conclusion that nicotine tolerance is produced by an increase in the ability of the body tissues to destroy the poison.

Before taking up the experimental evidence in support of this view, it may be well to discuss briefly a few of the points raised by Dixon and Lee in regard to earlier work along this line and especially those criticisms which they offer in regard to the experiments which one of us[3] carried out some time ago upon this subject.

These points are not brought up with any desire to enter into a controversy but in order to clear up any doubts which might have been raised in regard to the work of others as well as to the particular paper mentioned above.

In the first place, Dixon and Lee explain the failure of Gouget, Adler and Hensel and many of the earlier workers to obtain tolerance to nicotine in animals by the fact that they gave the nicotine by intravenous or subcutaneous injection and they add that "tolerance either cannot be obtained or is insignificant when such methods are adopted." This statement may be true but if so why do Dixon and Lee adopt these methods themselves when they desire to induce tolerance in animals? And not only do they adopt them but in their description of the effects of these intravenous and subcutaneous injections they say that the effects of the "later doses were a little less pronounced than the earlier." Was this diminution in effects due to tolerance? It would be quite possible were it not for the statement quoted above that the methods of administration employed are practically useless for this purpose. Moreover these animals which were rendered tolerant by methods which are condemned are used to demonstrate the mechanism of the production of tolerance. The only conclusion which seems justifiable is either that these animals were not tolerant or that the criticism of the work of Gouget and others is not correct.

Another point which may be considered is the test of tolerance which should be employed. Dixon and Lee do not consider vomiting a valid test, but at the same time it is rather hard to tell just what standard they selected. In the earlier subcutaneous administrations their rabbits showed tremors, twitchings, and signs of paralysis, while after intravenous injections they had convulsive movements and complete unconsciousness, all of which after the later doses are said to have appeared less pronounced than the earlier ones. Such a standard, however, lays itself open to the very serious criticism of being entirely too indefinite and as being too much a subject of opinion. Of especial interest in this connection is the testimony of Gouget who found that the intravenous injection of the infusion of tobacco into rabbits was always followed by convulsions which were always about the same where equal doses were given.

In addition to Gouget, Adler and Hensel report that nicotine in 1½ mg.

[2]Dixon and Lee: Quart. Jour. Exp. Physiol., v, 1912, 373.
[3]Edmunds: Jour. Pharm. and Exp. Therap., 1, 1909, 1.

doses, injected into the ear veins of rabbits produced convulsions which were always of the same intensity whether ten or a hundred injections had been given.

And this condition is exactly what would be expected if the nicotine tolerance is gained by an increased destruction of the alkaloid, as in intravenous injections no time would be allowed for such destruction.

Coming back, therefore, to the question as to whether the production of vomiting following subcutaneous injection can be considered a valid test, it may be said that that is perhaps a matter of opinion. It has the advantage of being perfectly definite and of equal importance it is the one symptom in man which denotes the possession of tolerance. The tyro in the use of nicotine rapidly shows his susceptibility to the drug by nausea and emesis and the fact that he has gained tolerance is shown later by the absence of these symptoms. Some individuals never gain tolerance as is shown by the fact that the use of tobacco is always accompanied by nausea. It would seem, therefore, that the use of this test for animals is perfectly logical and reasonable.

It is, of course, true as Dixon suggests that in case the drug is given by the stomach local irritation may produce vomiting, but a study of details of experiments I and II in the earlier paper referred to would convince anyone that local irritation was not the cause of the vomiting in the experiments. In Experiment I, for instance, with the doses being given under exactly the same conditions from day to day, there was what appeared to be a gradually increasing susceptibility to the drug so that whereas the cat had early tolerated 15 mg. doses of nicotine chloride it later vomited from doses of less than one-half that amount.

Finally, as a minor objection these writers appear to doubt whether any tolerance whatsoever was really obtained in the experiments on dogs in which large doses of nicotine were injected. They suggest that the failure to vomit after small doses is due to the depression which would follow the stimulation produced by the large toxic doses. This, of course, is a very important suggestion because if it is justified it would indicate the entire failure of the experiments with resulting erroneous conclusions. Here again, a careful reading of the paper and a study of the protocols to Experiments V and VI will show that such a condition as suggested was taken into consideration and that to avoid the effects of one dose being active at the time of the next dose, the injections were frequently made at intervals of two or three days but by experience it was found that this was not necessary "as the animals appeared to vomit just as frequently after a three or four day interval as after a rest of only one or two days."

In Experiment V one instance may be mentioned as illustrating this point. Fifteen mg. doses of nicotine were being given to a dog which had originally vomited after 12 mg. doses. On April 17th 15 mg. caused vomiting and on April 20th, that is three days later, 15 mgs. again caused vomiting but on the 22nd it caused no vomiting. Again, on the 24th, 27th, and 29th of the month, that is at either two or three day intervals, 15 mg. produced vomiting. We may repeat what was said in the original paper that the only conclusion that could be drawn from a study of the numerous injections made was that it seemed to make no difference in the results whether the injections were made at intervals of one or two days, or at intervals of three or four.

But granting that the criticism of Dixon and Lee in regard to the failure to vomit is valid, did not they, even after perceiving the supposed error, fall into the same error themselves? Their rabbits were injected on alternate days, and may not the apparent lessening of symptoms in their animals also be due to lack of recovery from previous doses? If it was possible in our series, it was equally possible in theirs.

Also the doses of nicotine which they gave were quite as large proportionately as those which were given to the dogs in the earlier work. In only one of the dogs was a 50 mg. dose exceeded and this for only four injections and such a dose of nicotine chloride for a hound weighing 15 Kg.[4] is not larger than a dose of 5 mg. of the alkaloid nicotine for a rabbit weighing less than one kilo. The Dixon and Lee paper also speaks of the lack of importance of the effects of "enormous doses used in the Edmunds experiments," but a little calculation will show that the average dose in Experiment V in the early part of the work was about 2 mg. of the nicotine salt (equal to 1.4 mg. nicotine) per Kg. and in only three injections was 4 mg. (2.8 mg. nicotine) per Kg. body weight exceeded, while Dixon and Lee's small doses were in the only protocol given 5 mg. of the alkaloid nicotine for a rabbit weighing 945 G. It is certainly true also that not one of the dogs showed symptoms anything like as severe as those which Dixon and Lee describe. Their rabbits showed twitchings, tremors, partial paralysis, and complete unconsciousness, while none of the dogs showed symptoms more severe than vomiting, trembling, and in some cases weakness in the hind legs. If, therefore, the "absence of vomiting" in the dogs does not indicate tolerance, as they suggest, neither do the effects which "appeared less pronounced" in their rabbits and, therefore, their rabbits were not tolerant and the whole theory of the mode of acquiring tolerance which they worked out must naturally fall to the ground. Such can be the only logical conclusion.

After this preliminary discussion which will serve the purpose of clearing up questions in regard to the reliability of previous work, the general content of the Dixon and Lee paper may be briefly summarized, taking for granted that the rabbits were rendered tolerant. After this had been accomplished by means of three subcutaneous and eleven intravenous injections of 5 mg. nicotine, the tolerant animal and a control were killed, and portions of the livers, freed from blood, were ground in a mortar with sand and saline solution and to this were added 2 c.c. of a one per cent solution of nicotine and 1 c.c. tuluol and the whole incubated for two and one-half hours. The solutions were then acidified, boiled, neutralized and filtered through muslin and paper, the two filtrates being of equal amount. The relative content of nicotine in the solutions was then estimated by means of the blood pressure rise which was produced in cats when equal doses were injected into the veins. Judged by this standard it was found that the liver of the animal which had been injected with nicotine destroyed a considerably larger amount of nicotine than did the liver of the normal animal. The writers further conclude that this destruction is brought about by the action of a ferment which is developed in the tissues of the rabbits under the influence of the injected drug.

[4]The shepherd dog used in Experiment IV of the early series weighed 15.6 Kg.; the black and white dog used in Experiment VI weighed 14.2 Kg.; and the hound used in Experiment V was about the same size.

These results are very interesting and important as they seem to indicate the mode by which the body protects itself when it is subjected to the influence of nicotine over a considerable period of time. It seemed to us therefore that inasmuch as it had been shown that it was possible to develop in dogs some degree of tolerance to the drug it would be of great interest to see whether in these animals it would be possible to demonstrate a mechanism of acquiring this tolerance similar to that described by Dixon and Lee for rabbits.

The method used in the effort to gain the tolerance was the same that was utilized in the earlier work, viz., to make the injections subcutaneously and starting in with a small dose to rapidly increase the dose until fairly large amounts were being given. The injections which were made at intervals of two or three days in the form of nicotine chloride, averaged in number from fifteen to twenty injections for each dog. It is unnecessary to give protocols for all the dogs which were treated, so only a condensed table will be given later in the article. It may be well to say that in no case were the symptoms any more severe than those described in the earlier paper. With the smaller doses the usual salivation and vomiting were present, accompanied in the case of the larger doses with dyspnea and some weakness in the extremities which was probably secondary to the repeated vomiting.

Ten dogs in all were used for this part of the work and the estimation of the nicotine destroying power of each liver was carried out upon cats in the same way as had been done by Dixon and Lee. After the dog had been killed, its liver was removed, freed from blood as completely as possible, and fifty grams of liver substance were placed with some sand in a mortar and to this were added 50 c.c. saline solution, 1 c.c. of toluol and 40 mg. of nicotine in the form of hydrochloride. After these had been ground up thoroughly the extract was incubated at 38° for three hours, then acidified, boiled, neutralized, strained and filtered. Fifty grams of liver substance from a normal untreated dog was treated in precisely the same manner, the two extracts being carried through at the same time.

When these extracts were completed they were used for injection into cats which were prepared for blood pressure estimation in the usual way. After being anesthetized with chloretone, cannulas were inserted into the trachea, into one of the carotid arteries and into the two external jugular veins. During the course of these estimations each animal was kept warm by means of an electric heating pad and given artificial respiration with heated air. The injections which were given at intervals of six minutes were given alternately, first the extract from the tolerant animal into one jugular followed by the control into the vein of the other side. The alternating doses were then continued until such relative amounts were found that the rise from each extract was alike. In some cases these doses were then doubled in order to confirm the ratio established by the smaller doses.

The following table will give the most important details of the experiments:

No. of Exp.	Wt. of Dog.	No. of Inject.	Initial dose of Nicotine given in form of the chloride.	Largest dose of Nicotine.	Relative strength of extracts as determined by effect on blood pressure.
1	10.9Kg.	16	5 mg.	50 mg.	1 c.c. tolerant—2 c.c. Control
5	6.6	14	2.5	50	1 c.c. " —0.5 c.c. "
6	7.5	18	2.5	53	1 c.c. " —0.75 c.c. "
7	8	17	26.5	53	1 c.c. " —1.4 c.c. "
8	14	17	26.5	58	1 c.c. " —1 c.c. "
9	15	15	26.5	58	1 c.c. " —1 c.c. "
10	7	23	13.3	57	1 c.c. " —1 c.c. "
12	8	17	13.3	58	1 c.c. " —1 c.c. "
13	7	12	13.3	46	1 c.c. " —1.15 c.c. "
14	6	20	13.3	58	1 c.c. " —0.75 c.c. "

A survey of this table shows first that out of the ten dogs which were subjected to ascending doses of nicotine in only three was there any evidence of an increased power of destruction of the alkaloid over that displayed by normal animals. These animals were Numbers five, six and fourteen. In them it seemed that the facts shown by Dixon and Lee as being true for the rabbit were also true for dogs. In four more dogs however (Numbers eight, nine, ten and twelve), could no such increased power be shown. Extracts of livers from these animals possessed no more power to destroy nicotine than did those prepared from livers of animals which had received no nicotine.

And as an offset to the first three mentioned there are still another three (Numbers one, seven and thirteen), the livers of which did not display as great destroying power as did those from normal dogs. The net results then would seem to be practically negative. The explanation of our results probably is that the livers of all dogs possess some power of destroying nicotine but that this ability is subject to great variations in different animals. If this power of destruction is due to a ferment action, the ferment must be increased in amount only very slowly and with great difficulty. That it is probably a ferment action is suggested by one of our experiments (No. 6) when a portion of the liver was boiled before being incubated—all the other particulars of the technic being the same as in the other cases—the results were as follows: 1 c.c. control—1.4 c.c. unboiled extract of tolerant—0.75 c.c. boiled tolerant. The ferment having been destroyed, the solution was relatively stronger even than the control which in itself had destroyed a certain amount of nicotine.

That some normal untreated livers are not devoid of the power of destroying nicotine is further shown by some of the experiments in which the control liver extract after having been compared with the tolerant, was compared with a nicotine solution of equivalent strength. Three experiments tend to show that the normal liver extract after incubation contained less nicotine than a control nicotine solution. Thus in one experiment (V) 1 c.c. of the normal control liver extract gave a smaller rise of blood pressure than .8 c.c. of the nicotine solution. In two more experiments (VI and XIV) 1 c.c. of the normal (control) liver extract gave rise in blood pressure equivalent to those produced by .75 c.c. and .67 c.c. of nicotine solution respectively.

In connection with our results it is interesting to note that Dixon and Lee

say that in three of their rabbits they failed to find any evidence of tolerance so it appears that the two animals do not differ very greatly in this respect.

The conclusions we would draw from our work are that the livers of all dogs possess some power to destroy nicotine and that the destroying agent is probably a ferment. It is possible that this ferment may be increased in quantity by the constant use of the alkaloid but our experiments do not furnish any support for this theory. The main difficulty appears to be that under normal conditions the destroying powers of different livers vary so much in strength that it is almost impossible to state that it has been increased by treatment.

HEXAMETHYLENAMINE AS A URATE SOLVENT, AND DIURETIC, AND ITS EFFECT ON THE REACTION OF URINE*

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ALONG with piperazine and some other methylene diamines hexamethylenamine (Bardet, 1894; Nicolaier, 1894) was originally proposed as a uric acid solvent. This alleged virtue Nicolaier claims to have observed quite incidentally. He noticed that in certain urines to which formaldehyde was added as a preservative the deposits which had formed on standing disappeared, even in the presence of hydrochloric acid. This he attributed to the formaldehyde. Therapeutic possibilities were at once suggested, and hexamethylenamine as a non-irritating formaldehyde compound was proposed as the ideal drug. Its antiseptic properties were also soon recognized, and these have continued to claim a deservedly greater popularity (see Hanzlik and Collins, 1913, and appended literature). However, a considerable medical clientele appear still to possess faith in its supposed virtues as a urate solvent. This is supported and fostered by interested manufacturers, whose exaggerated claims, however, have served to dampen the enthusiasm for these hoped-for virtues.

The mode of action of hexamethylenamine as a urate solvent has never been quite definitely understood. In fact it has been vaguely attributed sometimes to its chemical properties, sometimes to certain alleged physiological effects. For instance, owing to the fact that formaldehyde can combine with uric acid to form soluble compounds, and since hexamethylenamine was supposed to liberate formaldehyde in the body, this has been a favorite explanation with many. Then again hexamethylenamine and uric acid are said to combine directly with the formation of a salt, called hexamethylenamine urate. Finally, diuresis due to some supposed physiological action of hexamethylenamine, also claims its adherents. There are reasons for supposing that neither one of these explanations is necessarily correct, or quite adequate in view of other attending circumstances. A review of the important literature was purposely made with the object of ascertaining what claims, if any, can be made for hexamethylenamine as a urate solvent.

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This review was prepared at the request of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

The results of this search are sufficient to indicate the negative value of hexamethylenamine in this direction. Unfortunately the nature of such studies as here reported is surmounted with numerous difficulties, and it cannot be said that the investigations undertaken to elucidate these have been always characterized by the most careful scientific scrutiny. Much of the confusion has resulted from a failure to realize and appreciate the chemical and solubility characteristics of uric acid and its salts and their behavior in the organism. Since these fundamental considerations cannot be safely overlooked in judging of the real value of hexamethylenamine as a urate solvent, it was deemed necessary to present first a resumé of them. Following this the effects of hexamethylenamine will be considered.

Uric Acid and its Salts.*

Uric acid is a weak dibasic acid and can form three series of salts: (1) neutral or dibasic, normal or secondary urates; these are formed only by strong alkalies and cannot occur under natural conditions, being decomposed by carbon dioxide and even water; (2) acid urates, or monobasic, primary or biurate; these are neutral to litmus; (3) quadriurate; hemibasic or tetra urate, a mixture of uric acid and acid urate (Kohler, 1911); according to Ringer and Schmutzer (1912) a "solid solution of uric acid in acid urate which can be formed at body temperature, but which is unstable on cooling and thus separates uric acid."

Solubility.—Free uric acid is very little soluble in water. According to His and Paul (1900) pure water dissolves at 18° C. only 25 mgms. per liter=1:40,000; at 40° the solubility is doubled. Alkalies, even sodium bicarbonate and phosphate, transform part of the uric acid into acid urate and therefore increase its solubility. Free acids lessen solubility since they decrease the ionization (His and Paul, 1900). Addition of free uric acid to urine may precipitate a part of uric acid originally in solution (Voit) presumably by acting as a crystallizing nucleus. The solubility in serum is much greater (500 mgms. per liter) than in water (Bechhold and Ziegler, 1909). This is probably due to the colloids and it is probable that the colloid constituents of urine also contribute to the solubility of uric acid and urates (Lichtwitz, 1910). Lichtwitz, however, denies an important role to urochrome as was claimed by Klemperer, and finds that practically all the uric acid of urine is in true solution (not colloidal) for it dialyzes easily from urine. Ruedel (1892) claimed urea to have an important solvent action, but Ritter (1897) finds this very limited. The acid sodium phosphate precipitates and liberates uric acid. This is counteracted by the presence of the alkaline phosphate, and is of interest in connection with urine.

According to Allan and Bensch (1848), the solubility of the different urates in water is about as follows: lithium 1:350; potassium 1:800; sodium 1:1200; and ammonium 1:1600; alkaline earths 1:4000 to 6000. Excess of alkali or other salts decreases the solubility by "salting out" in proportion to their concentration (Roberts, 1896; Ritter, 1897). The salts of plasma reduce the solubility to 1/10 of that in water (His and Paul, 1900). Solid sodium acid urate occurs in two forms; when freshly precipitated is gelatinous, colloidal or amorphous, later becomes crystalline. The colloidal form is more easily soluble (Bensch, 1845). Gudzent (1909) claims that two forms exist, the more soluble lactam and the less (2/3) soluble lactim.

Occurrence in the Body and Tissues.—Uric acid as such does not exist in the blood. It appears in the form of the acid urates in solution in the blood and tissues and as solid biurate deposits in gouty tophi (Ritter, 1897). The free acid and acid urate appear in scanty or acid urine. Being crystalline the urate deposits act as powerful mechanical irritants producing some of the local phenomena of gout. These can be simulated by the subcutaneous injection of suspensions of acid sodium urate and in birds by subcutaneous injection of chromates or ligation of the ureters. The cause of the abnormal appearance of urate deposits in gout is not understood. Recently, Benedict (1915) has obtained results with ox blood which seem to indicate that uric acid appears in some combined non-excretable form in the blood corpuscles, besides the urate in the plasma, which would be the excretable form.

*The material in small print under this heading was in part abstracted from the manuscript of Professor Sollmann's revised Textbook of Pharmacology.

Effects of Solvents on Urate Deposition in Tissues.—The mobilization or removal of urates from the tissues in such conditions as gout, and possibly others, would be desirable; also, to prevent the formation of urate deposits in tissues and urine. What has been said concerning the chemical properties of the urates serves to indicate the difficulties of doing this.

The study of solubility is greatly complicated by small quantities going into solution, polyvalence, formation of colloidal solutions, supersaturation and the influence of small quantities of impurities, decompositions, etc. (His and Paul, 1900). Alkalies dissolve a limited amount of free uric acid by conversion into the more soluble acid urates and that limited success is confined to urine.

When all the uric acid exists as acid urate, as it does within the body, the addition of alkali can have no further solvent action. On the contrary, excess of alkali or other salts tends rather to decrease the solubility of the urates. It is evident, therefore, that no direct solvent action can occur in urate tophi (Ritter, 1897). This inefficiency has been confirmed clinically. If alkalies have any effect, which is doubtful, it would be solely by their diuretic action. Loghem (1907) claimed that hydrochloric acid is more useful for preventing formation of urate deposits in animals, presumably by lessening the "salting out." Ritter (1897) had a similar idea, but this was not confirmed by Staal (1908).

The conditions for solvent efficiency of alkalies is much better in acid urine. This contains not only acid urates, but also free uric acid often in saturated solution. When urine cools, it becomes more acid and under other conditions solutions may become supersaturated. The uric acid will then precipitate alone or in combination with a part of the acid urate (quadriurate). This can be prevented either by making urine more dilute (diuresis) or by making it less acid. Alkalies according to Buchheim (1853) act in both directions. Too high concentrations of alkali as other salts render acid urate less soluble, but in practice the administration of alkalies does not raise the total concentration of urine especially if the water is taken freely. Alkaline urines would also dissolve actually precipitated uric acid, for the urine, after the administration of alkalies, is able to dissolve more uric acid than before (Haskins, 1914). With the practical limits of the alkalinity of the urine, however, the solution is so slight that it has little effect on formed calculi. The introduction of alkaline solutions into the bladder has not been more successful (Buchheim, 1853).

It is sometimes stated that the solubility of urates is improved by the administration of potassium or lithium salts. This is true of pure solutions, or when sodium urate (or urate stones or tophi are treated with lithium carbonate), but it cannot occur in the presence of an excess of sodium; for according to ordinary chemical laws, when several bases compete for an acid, it is the least soluble salt that is formed. Since there is always an excess of sodium ions in the blood and urine, the lithium and potassium would have no chance to exert their solvent action. The same reasoning applies to certain organic bases, such as the ethylen-amines (also, hexamethylenamine), piperazine and piperidine. These also would be unable to compete against the sodium. Moreover they are excreted imperfectly, so that their efficiency in the urine is probably inferior to that of ordinary alkalies (Haskins, 1914).

The relation of reaction and volume of urine to solubility of uric acid was studied by Blatherwick (1914). He found that the extent of solution of uric acid in urine appears to be chiefly a function of hydrogen-ion concentration. There was a striking regularity in the deposition of uric acid in urines having a greater hydrogen-ion concentration than 7.0 (neutrality), while alkaline urines showed an increased capacity to dissolve. Very acid urines were generally found to be supersaturated and neutral specimens were nearly saturated. Ordinary variations in urinary volumes exerted an insignificant influence on uric acid solubility. These changes can be effected by appropriate variations in a dietary composed of commonly used articles.

Excretion of Uric Acid (Aside from Dietary Influence).

The various factors in the quantitative excretion of uric acid by the kidney, using the accurate colorimetric method of Folin, has been studied by Robertson (1914) with no definite results. The injection of pituitary, saline, barium chloride, caffeine and piperazine and sodium sulphate produced no changes in uric acid excretion which were sufficiently independent of urine flow to suggest any specific effect of the agents used. In fact it could not be definitely es-

tablished whether increased water excretion or change in the blood flow through the kidney is primarily responsible for the parallelism between increase in urine flow and uric acid excretion. Certain of the results indicate that uric acid excretion varies independently of the urine flow, and that increased uric acid excretion does not necessarily lead to much increase in the amount of urine. Preti (1915) has recently shown that the increased intake of carbon dioxide (respiration) increases the elimination of uric acid. So far as drugs are concerned, it can be stated that atophan (Folin and Lyman, 1913; Haskins, 1913), salicylate (Denis, 1915), and colchicum produce a definite increase in the excretion of uric acid into the urine; alcohol is somewhat doubtful (Jackson and Blackfan, 1907). According to Abl (1913) diminution in the excretion is brought about by calcium salts, barium sulphate and bismuth nitrate, whereas mustard, arsenic, colchocine, thorium, sulphur, santonin, glycerin, choline, chloral, neurine, strontium and piperazine increase elimination. These drug actions may be explained by increase in renal permeability in some cases, or disturbances in the systemic circulation in others; increased oxidation may also play a part.

The role of the digestive glands in the production of endogenous uric acid has recently been emphasized by Mendel and Stehle (1915). Drugs which augment glandular activity, such as pilocarpine, brought about an increased excretion, whereas atropin (antagonist) lessened. Mechanical work of the intestine alone did not influence the production of uric acid.

This rather lengthy preliminary survey is considered necessary for a proper appreciation of the properties of uric acid and the urates and the various conditions which influence and underlie their behavior in the body. It will suffice to indicate the complex nature of any problem which pretends to the analysis of the solvent properties and excretory powers of a given agent for uric acid or urates. From what has been said of the influence of the various factors governing the solubility and excretion of urates, and the probable chemical and physiological effects which can be produced in this direction by hexamethylenamine, its chances of success as a uric acid solvent would be rather limited. There are certain properties which hexamethylenamine must exhibit if it is to produce the desired effects. These are: (A) As a urate solvent: (1) a urine containing hexamethylenamine must take up distinctly more uric acid than do normal urines of corresponding acidity and concentration; (2) to be of value in calculi, gravel, and removal of free uric acid, it must dissolve these more readily than urine itself without the influence of concentration and alkali. (B) For urate elimination: Administered internally a definite increase in elimination of urates, uninfluenced by other factors, such as diuresis, diet, drugs, etc., must be demonstrated. The available facts in the literature will now be judged on the basis of these conditions, and unfortunately, it may be intimated, with not always the best results for hexamethylenamine.

Addition of Hexamethylenamine to Pure Urate.

According to the experiments of Tunicliffe (1897) a 3.5% aqueous solution of hexamethylenamine dissolved 0.7% of added uric acid at 17° C.; 3.3% at 36° C., by prolonged heating on a water-bath. The solution of hexamethylenamine was acid to litmus. Hence it is probable that formaldehyde was present, and caused uric acid to go into solution. Casper (1898) found that 300

Gms. of water dissolved 0.024% of uric acid in 5 days at room temperature, none at 37°, whereas a 1.3% hexamethylenamine solution dissolved 0.18% in 5 days at room temperature, and 0.016% at 37° C. Nicolaier (1899) observed that hexamethylenamine solutions dissolve more uric acid than water alone at 18° C., and this was augmented by about seven times at 37° C. According to Tunicliffe and Rosenheim (1898) solutions of uric acid in hexamethylenamine gelatinize on cooling, behaving in this respect like colloids, but differing from them by being able to dialyze. The experiments of Nicolaier (1899) indicate that while hexamethylenamine dissolves uric acid and urates at body temperature, it is less active in this respect than piperazine. Heinz (1907) and Penzoldt (1910) claim that hexamethylenamine *in vitro* has considerable solvent action for uric acid, but no evidence is offered for this. Ortowski (1910) reports that the solvent properties of hexamethylenamine for uric acid at 37.5° C. are slight, but that passage through the body imparts to the urine marked solvent properties. Stevens and May (1911) found that a 1.5% solution of hexamethylenamine was only a fair solvent for added uric acid, requiring considerable agitation to effect solution. Only 0.035% of uric acid was dissolved, and on prolonged standing (1 to 14 days) in contact with hexamethylenamine no destruction or decomposition of the uric acid was demonstrable. As compared with hexamethylenamine the solvent properties of lycetol were less, but that of piperazine greater. Kobert (1906) states that hexamethylenamine *per se* forms a compound with uric acid from which uric acid cannot be regenerated. This does not seem to be the common view. Whatever solubility takes place is said to be due to liberated formaldehyde with the formation of diformaldehyde uric acid (Tollens; Nicolaier). This is believed to be the case by Haskins (1915), whose work shows definitely that hexamethylenamine in concentrations (0.05 to 0.1%) comparable to amounts excreted after therapeutic doses has no demonstrable influence on the solubility of uric acid under conditions resembling urine. In higher concentrations (1%) hexamethylenamine dissolved small, but distinctly demonstrable, quantities of uric acid with the liberation of formaldehyde. The experiments were performed by adding uric acid to mixtures of di- and monosodium phosphates, since the degree of reaction plays an important part in the solubility. Haskins found that 0.1 to 5% solutions of hexamethylenamine in plain distilled water dissolved 0.035% to 0.25%, respectively, of uric acid, but he observed that the acidity (hydrogen-ion concentration) of ordinary distilled water (due to CO_2) is sufficient to cause liberation of formaldehyde from the hexamethylenamine. The solubility, therefore, was not due to hexamethylenamine *per se*. Water alone dissolved only 0.0084% of uric acid. Another instance of how a relatively small degree of acidity tends to facilitate the uric acid solubility of hexamethylenamine as compared with a small degree of alkalinity was observed by Haskins as follows: A 1% solution of the drug in a phosphate mixture of a hydrogen-ion concentration of 7.2 (alkalinity) dissolved a smaller amount of extra uric acid than a 1% solution of the drug in a phosphate mixture of the same concentration, but of greater hydrogen-ion concentration, 6.8 (acidity).

Why hexamethylenamine dissolves extra uric acid in alkaline mixtures is explained by Haskins (1915) as due to the fact that the acidity of the solution gradually changes as uric acid is taken up. For instance, the filtrate from

a 7.2 (alkaline) phosphate mixture after shaking with uric acid had an acidity of 6.8. In consequence of this, formaldehyde was finally liberated in the hexamethylenamine phosphate mixture and dissolved extra uric acid, whereas in the same acid solution (6.8) formaldehyde was being liberated during the entire time of shaking. While the time for liberation of the formaldehyde may be relatively short, this is compensated for by the greater concentration of the drug.

Concerning the combining power of formaldehyde with uric acid, it may be further said that formaldehyde combines with uric acid to form loose and in part at least, very easily soluble compounds in water. This was investigated by Weber, Pott, and Tollens (1897), but it is not quite clear (although conceivable) whether this combination takes place under conditions of the body (i. e., in urine) when formaldehyde is liberated from hexamethylenamine. These investigators prepared formaldehyde compounds by adding uric acid to 40% formaldehyde at 100 to 110° C. Two sets of products, which differed in elemental composition, were obtained, and formaldehyde from one of these (di-formaldehyde) was liberated by prolonged boiling only.

In Nicolaier's (1906) contribution on compounds of uric acid with formaldehyde, nothing of importance is added to the subject. In their work both Tollens and Nicolaier make use of 40% formaldehyde in the preparation of these uric acid compounds. In no case have there been used such concentrations of formaldehyde as can appear in the urine after administration of hexamethylenamine. Final judgment as to the possibilities of formation of such formaldehyde uric acid compounds must be deferred until these have been investigated under conditions of the body.

The degree of the reaction (H^+conc.) is the most important factor in the problem of urate solubility and, unfortunately, was practically entirely overlooked by all workers previous to Haskins (1915). This greatly limits the value of their work. As will be seen later, it is the paramount factor of importance in urinary solubility, which will shortly be considered.

The nature of the evidence just cited permits us to conclude that the solubility influence of hexamethylenamine *per se* when added directly to pure uric acid is practically nil, particularly if the influence of reaction, salts, temperature, and other factors are discounted. When formaldehyde is liberated, a small but practically insignificant quantity of uric acid is dissolved.

Addition of Hexamethylenamine to Urate Calculi.

The observations on this are extremely limited. According to Nicolaier (1904), the action of hexamethylenamine on uric acid concretions is slow and difficult to judge. His own observations are limited to urine, and will be discussed later. Casper (1897) states that hexamethylenamine solutions, no matter how strong, hardly dissolve uric acid calculi any more than ordinary water, and at room temperature solution proceeds somewhat more slowly than at body temperature. This is confirmative of some preliminary experiments reported by Loebisch (1897). Casper also observed that as a solvent for calculi, hexamethylenamine is no more effective than lysidine or piperazine or other agents recommended for that purpose. Drake-Brockmann (1900) found that weak and concentrated solutions of hexamethylenamine produced no change in the weight of small portions of immersed calculi even after incubation for several days.

While the paucity of data must be admitted, the general tone of the existing observations is unfavorable to hexamethylenamine as a solvent for calculi.

Solvent Action of Hexamethylenamine Urines on Added Uric Acid and Urate.

Nicolaier (1899) observed that urines which previously did not dissolve added uric acid at body temperature, did so when they contained hexamethylenamine. However, Nicolaier reports but few results, and the influence of the reaction of the urines was not studied. Casper (1898) performed a single experiment with 200 grams of urine of a man who received 3 grams of hexamethylenamine, and found that in 5 days at room temperature 0.027% of added uric acid was dissolved, and 0.14% at 37° C., but no reference is made to the reaction of the urine during this time, and Casper, rightfully, does not lay stress on this observation. Tunicliffe and Rosenheim (1898) observed that when hexamethylenamine (0.2 Gm.) was added to urine (100 c.c) kept at body temperature for two and a half hours, about 6 times as much of the added uric acid was dissolved as compared with the same urine without the drug. Here again nothing is stated concerning the reaction or concentration of the urine, and this same criticism applies to the claimed superiority for piperidine and lysidine, which according to these authors would be about thirty times that of hexamethylenamine.

Haskins (1915) found that when hexamethylenamine (0.05% to 0.1%), in quantities comparable to therapeutic, was added to normal urines, no more added uric acid was dissolved than in the same urines without the drug. Strong concentrations (1%) of the drug dissolved more uric acid than the urine itself.

In connection with this the observations of Tunicliffe and Rosenheim (1898) on the solubility of serum for sodium biurate may be mentioned, for, so far as I know, they represent the only data of their kind in the literature. Tunicliffe and Rosenheim found that when hexamethylenamine (0.1 Gm.) was added to beef serum (100 c.c.) containing sodium biurate, the solubility of the biurate was increased over that of serum alone. With serum alone, the solubility was 1:60,000. When hexamethylenamine was added, this was increased to 1:14,000. If the solubility of biurate in serum alone is given, the value of 1 of that serum plus hexamethylenamine would have the value of 4.5. Piperidine gave 4.7, but lysidine and piperazine less than hexamethylenamine. The quantity of biurate in the serum originally present was very small, about 0.00029%. Although the reaction of the serum was not observed, these experiments appear to have been otherwise properly performed. If the serum possessed a neutral or very slightly alkaline reaction (even after incubation), then it appears that hexamethylenamine itself would possess some solvent influence on biurate, which, although an acid salt, would not influence the reaction sufficiently. On the other hand, if the serum solution became truly acid, as it frequently the case with incubated body fluids, then formaldehyde was liberated and would explain the solubility to some extent at least. A confirmation of these experiments is necessary before this feature of urate solubility by hexamethylenamine is accepted as proven.

Effect of Administration of Hexamethylenamine on Uric Acid and Urate Solubilities.

Nicolaier (1895) claimed that an individual passing a urine which on incubation and on standing deposits urates, will not show this if hexamethylena-

mine is given in sufficient dosage, and that the urine loses its uric acid solubility when the administration of hexamethylenamine is stopped. A case of leukemia is cited in which the urine exhibited urate sediments on all days except when hexamethylenamine was administered. The dose of hexamethylenamine was 6 Gms. per day. This effect was not thought to be due to increase in diuresis, since dilution of the same urine did not alter the quantity of sediment; also not to concentration, since the specific gravity was the same. The reaction of the urine was said to be unchanged qualitatively (acid), but no observations on the reaction in a quantitative manner were made, nor on the quantity of uric acid. In fact solution of uric acid in the incubated hexamethylenamine urine progressed slowly for several days; this being especially true of urate concretions.

Smaller daily doses of hexamethylenamine (1 to 1.5 Gms.) also caused urine to take up added uric acid. However, this varied within wide limits, and was greatest usually with the least concentrated urines, especially if diuresis increased during the administration of the drug. Nicolaier states that the reaction (acid) was not changed during the medication, however the degree of the acidity from time to time was not ascertained.

Nicolaier states that urine at body temperature could not dissolve concretions, but after sufficiently large doses of hexamethylenamine (1 Gm. three times a day), the urines at 37° began to dissolve the concretions during the first twenty-four hours, and solubility progressed slowly, so that in a few days only the organic protein-like structure remained undissolved. The solution property was lost when hexamethylenamine was stopped. A case from such a series of observations is cited. The degree of acidity of urine was not determined, and diuresis is excluded on the grounds that the same urine, when diluted and incubated at 37°, did not dissolve the concretion.

In his attempt to explain the supposed hexamethylenamine solubility of urate, Nicolaier admits the importance of diuresis. However, this is not thought to be the only cause, since solubility of uric acid occurs without it. Reaction of the urine is considered important in creating a favorable condition for solution. However, since the reaction remains acid and practically unchanged during hexamethylenamine medication, and hexamethylenamine itself is a weak base, and cannot lower the acidity, Nicolaier thinks that too much stress should not be laid on this, and prefers to regard solubility as an action of the drug itself.

The mechanism of this action is attributed to liberated formaldehyde, which is said to form very soluble compounds with uric acid, this being more true at body than room temperature, since heat facilitates the decomposition of hexamethylenamine. No experiment of his own is cited by Nicolaier for this theory. The presence of formaldehyde was not demonstrated, nor is the possibility of the likely quantity necessary for union with the added uric acid indicated.

Casper (1897) states, that after numerous unsuccessful experiments with other reagents, he does not believe the favorable reports of Nicolaier on the solubility of hexamethylenamine for concrements. Casper gave hexamethylenamine in large doses to patients with uric acid, gravel or stone, without observing any positive evidence of the solubility of the concretions. Casper observed that urine during and after administration of hexamethylenamine dis-

solved uric acid just as poorly as urine of the same subject without the drug.

The supposed beneficial results in a case of pyelitis calculus urica on a purin-free diet reported by Loebisch (1897) are attributed to diuresis and formaldehyde liberated in the blood. However, Loebisch falls into error regarding the presence of free formaldehyde in blood. That such does not occur is universally admitted (see Hanzlik and Collins, 1913; McGuigan, 1914). He also states that hexamethylenamine dissolves sodium acid urate at room temperature very slowly, but very rapidly on incubation.

Nicolaier (1904) asserts that the urate solvent property of hexamethylenamine is not due to the drug itself, but rather to the liberated formaldehyde. Nicolaier states again that 1 to 1.5 Gm. of hexamethylenamine has uric acid solvent properties, especially in urate concretions.

The observations of Rosenfeld and Orgler (1896) on the effect of administration of hexamethylenamine are too few and the method for estimation of uric acid unreliable. Their conclusions, that hexamethylenamine acts by lessening formation and improving solubility of uric acid, are unjustifiable, since the results can be explained on the basis of differences in diuresis and sudden changes in diet, which during the experimental observations (when hexamethylenamine was administered) consisted of calves' sweetbread (500 Gm.).

According to Ortowski (1900) very marked uric acid solvent properties are exhibited by the urine only after passage of hexamethylenamine through the body, the precipitability of uric acid is lessened, and in this as well as direct solubility, it is superior to piperazine, uricedine, lysidine and bicarbonate. However, Ortowski's observations are extremely limited, and the results do not warrant the conclusion. The reaction and concentration of the urine were not taken into account. Rosenfeld and Orgler (1896) claim that hexamethylenamine not only increases the solvent properties of urine, but sometimes lessens the excretion of uric acid. Klemperer (1904) claims to have confirmed an observation of Nicolaier that after the administration of 6 grams of hexamethylenamine the 24-hour urine had 1/5 of the entire amount of uric acid present in the form of non-precipitable formaldehyde compound.

Haskins (1915) made a large number of observations, with strict control of the reaction of the urines. Since he is the only investigator who has taken the necessary precaution, the value of his results outweighs all the others. He found that urines after therapeutic doses of hexamethylenamine had no greater solvent power for uric acid than normal urine of similar concentration, or of similar hydrogen-ion concentration. It appeared that when excessive doses (4 grams) of hexamethylenamine were given to normal individuals, the solvent power of the urines was somewhat greater than was secured with normal urines of the same hydrogen-ion concentration. Similar effects, however, were more easily and just as effectively secured by the administration of therapeutic doses of alkaline diuretics and sodium bicarbonate.

From all this it is seen that the older observations on the administration of hexamethylenamine as urate solubility are contradictory. This is due to the fact that such factors as degree of reaction, concentration of the urine, diuresis, dietary influence, the uncertain amount of hexamethylenamine present and formaldehyde liberation were either not, or imperfectly considered. More recent, and better controlled observations which give due consideration to these factors seem to nullify any importance that was attached to the increased solvent properties of hexamethylenamine urines for uric acid above and beyond that of urine itself. An exception must be made to the use of extra large doses of

the drug in which case there appears to be a solvent effect, though slight and practically unimportant, exerted beyond that of the urine itself. It is not absolutely certain that such large doses of hexamethylenamine are devoid of harmful effects. Moreover, by means of alkaline diuretics, in ordinary therapeutic quantities, just as efficient urate solvent properties are imparted to urine. The use of these is also more economical.

Effect of Hexamethylenamine on Diuresis.

Aside from statements of opinion or impression, I have found no scientific observations on this in the literature. The following are cited for whatever worth they may possess.

Increased diuresis following the administration of hexamethylenamine is claimed by Nicolaier (1895; 1904), Lilienthal (1900), Flexner (1895), Impens (cit. Nicolaier, 1904), and Seifert and Müller (cit. Nicolaier, 1904). Nicolaier claims that 1 Gm. doses were diuretic. In order to obtain efficient diuresis Lilienthal recommended as much water for the patient as could be taken comfortably. Seifert and Müller and Impens state they obtained diuresis with hexamethylenamine citrate ("helmitol"). Nicolaier denies having observed an increase in diuresis after "helmitol," but does not deny the probability of its occurring in some individuals.

Thompson denies the diuretic action of hexamethylenamine; also Strauss and Seibert (cit. Nicolaier, 1904) for "helmitol."

It is evident that nothing definite can be concluded from the sort of evidence available. When no reference is made to fluid intake, no conclusions can be drawn from the urine output. The most that can be said is that the opinions are conflicting.

Effect on the Reaction of Urine.

The only scientific evidence available is that reported by Haskins (1915) with the addition of hexamethylenamine to urine. He found that this did not alter the true reaction (hydrogen-ion concentration) of the urine, whether acid or alkaline. After administration of hexamethylenamine Casper observed that the reaction (by titration) of urine remained unchanged. In a study of the effect of different organic bases related to hexamethylenamine on the excretion of uric acid, Haupt (1895) found that the solvent property which the urine possesses appears to be dependent on the alkalinity present, and not on the direct action of the organic base excreted into it.

On the other hand, Cumiston (1898) believes hexamethylenamine lessens acidity. The general tendency of clinical writers has been to claim a change from alkalinity (ammoniacal) to acidity in the reaction. Such claims, and without offering any experimental evidences whatsoever, are made by Nicolaier (1897), Flexner (1895), Greene (1899), Thompson (1899), and Wilcox (1898).

Nicolaier (1897), for instance, makes the statement that urine, after the administration of 1 to 1.5 Gm. of hexamethylenamine, will become ammoniacal at room temperature, but remains clear and retains an acid reaction if incubated at 37° C., and ammoniacal fermentation does not occur even if in a few days a few drops of an ammoniacal urine are added to it. Nicolaier also states that

hexamethylenamine, in doses of 1.5 Gm. per day, rendered ammoniacal urine acid again in a short time. These statements undoubtedly have helped to crystallize the opinions of successive writers on this subject. However, it cannot be said that it has resulted in anything but the perpetuation of faulty evidence.

An example of this is suggested in the statement of Thompson, "It renders a foul-smelling, alkaline urine sweet and acid, and is therefore an acidifier."

In some cases (Flexner, 1895; Greene, 1899), a brief statement endorsing this alleged property is made.

Nicolaier (1904) denies to "helmitol" any influence on the reaction of the urine.

Effect on Calculi.

Flexner (1895) and Nicolaier (1904) state that calculi are dissolved after administration of hexamethylenamine. Wood (1902) states that hexamethylenamine is less active than piperazine and has no value for calculi. The same is claimed by Casper (1897), who made some observations with the administration of hexamethylenamine. However, these statements are unsupported by scientific evidence. Moreover, they are contradictory, and from other evidence it can be safely stated that the favorable claims reported by some for hexamethylenamine as a calculi solvent are unjustifiable.

Summary.

A resumé of the chemistry and the behavior of various solvents toward uric acid and urates is given, and indicates very remote possibilities of chances of success of so-called urate solvents under conditions of the body.

Urate or uric acid solubility is a matter which is concerned largely with the degree of reaction (hydrogen-ion concentration) and the concentration of fluids. There is no available evidence in the literature to show that hexamethylenamine can influence these favorably.

Recent and reliable evidence shows definitely that hexamethylenamine *per se* in small quantities, or therapeutic doses, imparts to urine no demonstrable urate solvent qualities.

Slight and practically negligible uric acid solvent effects are imparted to urine by excessive doses of hexamethylenamine, and in this, the effects of the common alkaline diuretics are pharmacologically and economically superior.

There is no evidence that hexamethylenamine can dissolve urate calculi.

Regarding the efficiency of the class of drugs called "urate solvents," the statement of Fränkel (1906), being quite to the point and appropriate, may be cited, namely, that no substance has yet been discovered which would form either soluble or easily oxidizable compounds with uric acid under conditions of the body.

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GONOCOCCUS COMPLEMENT FIXATION: A NEW LIPOID ANTIGEN*

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IN a paper before the Section on Genito-Urinary Diseases at the San Francisco meeting of the American Medical Association, one[1] of us made a preliminary report on the use of the fats of the gonococcus as antigen in gonococcus complement fixation tests, in which the superiority of an alcoholic solution of these fats over watery extracts was shown. This observation grew out of a study[2] of the biochemistry of the gonococcus, wherein the importance of the lipoid constituents became apparent. This article presents in detail the conclusions summarized in the preliminary report.

Antigens.

In all the tests a good commercial watery antigen (Parke, Davis and Company) was used parallel with the Warden antigen. The latter was prepared in such manner that each 1 c.c. of absolute alcohol contained in solution 0.001 gram of gonococcus fats as fatty acids. To this was added one-third volume of a one per cent alcoholic solution of cholesterol. Alcohol and ethereal extracts of gonococcus substance are of no value as antigen. The gonococcus substance from which the fats were separated was obtained from cultures of twenty different strains. From the standpoint of immunity, the question whether the fatty antigen may not contain a proteid portion, or "rest," of the gonococcus is an important one, as well as that whether the body of the patient may not be sufficiently sensitized to the protein of the cocci present during the disease, but neither need concern us at the present time.

Technic of the Complement Fixation Tests.

We have used the sheep-rabbit-hemolytic system in which the serum was active in dilutions of 1-10000, with fresh guinea-pig complement and thrice washed sheep cells. The titer of the antigens and hemolytic serum was made frequently in the usual manner. The commercial antigen was used in the maximum quantity, permitting complete hemolysis in a given time, and the Warden antigen in one-half the lowest inhibiting dose. The serums and other materials were measured by drops from standard pipettes. When set up, the tubes contained, in addition to the antigens, one drop (0.02 c.c.) of the patient's serum, one drop of complement and sufficient salt solution to equal 1 c.c. After the first incubation of one hour there were added to each tube ten drops (0.2 c.c.) of a five per cent suspension of sheep cells, and an equal quantity of diluted hemolytic serum, representing two units of hemolysin, that is, twice the quantity sufficient to produce complete hemolysis in thirty minutes. The tests were invariably controlled by known positive and negative sera, and separate tubes for each ingredient. Particular caution was used in the titration of the com-

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[1]Warden, C. C.: Vaccine Treatment of Gonorrhea, Jour. Am. Med. Assn., Dec. 11, 1915.

[2]Warden, C. C.: Studies on the Gonococcus, Jour. Infect. Dis., May, 1916.

mercial antigen as it was observed that individual samples varied with time, and that different lots varied widely in anticomplementary power, although bearing the same unit quantity on the labels. The alcoholic antigen has not varied perceptibly in six months.

Results of the Tests.

The subjoined tables show the outcome of 423 tests upon the sera of 367 persons. Complete inhibition of hemolysis, a positive reaction, is indicated by the sign ++; partial hemolysis, still a positive reaction, by +. Doubtful reactions are shown by ±. We have regarded them as positive reactions as they have been not uncommon in the early and late stages of gonorrhea, and have not appeared in other conditions.

The list includes tests upon the sera of fifty normal individuals of all ages. In no case was the result other than negative. There are included also over one hundred cases of disease other than gonorrhea, in which a certain proportion of positive reactions was obtained, and in which evidence of gonorrhea could not be eliminated, a fact which speaks for the value of the test. Inasmuch as the Wassermann reaction depends on lipoidal properties of the antigen, particular attention was paid to luetic sera, but without detracting from the specificity of the gonococcus antigen.

The divisions made of the material in the tables are purely arbitrary, and have been made merely for convenience in classification.

The presence of gonococcus was determined where possible by culture methods and by *characteristic* smears.[1]

The abbreviations P. and W. refer to the commercial antigen and the Warden antigen, respectively.

TABLE I.

Diseases Other Than Gonorrhea.

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	DISEASE.	DATE.	P.	W.
1.	F. W. L.	39 yrs.	Gc. 1906, Clin. cure.	Colon infect. of kidney.		–	–
2.	J. R. S.	30 yrs.		Syphilis. Wass. +		–	–
3.	J. L.	28 yrs.		"		–	–
4.	E. N.	50 yrs.		"		–	–
5.	A. B. H.	No. 31, 20 yrs.		"		+	+
6.	A. H. (Mrs.)	?		Psoriasis		–	–
7.	S. S. F.	?	Gc. 1903-1912. Clin. cure.	Syphilis. Wass. +		–	–
8.	D. F. M.	34 yrs.	Doubtful. No recent Gc.	Rupture of urethra, 1895.		++	++
9.	B.	33 yrs.	Indefinite smears, 1913.	Syphilis (Wass. +)	5/18/15	++	++
	B.	33 yrs.	" "	" "	5/24/15	++	++
10.	H. H.	39 yrs.		"		–	–

[1]The writers do not regard the Gram stain essential. Reliance is placed more upon the appearance of uniformly prepared smears stained with Loeffler's blue, in which the size, morphology and intracellular grouping constitute the characteristic picture.

DISEASES OTHER THAN GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	DISEASE.	DATE.	P.	W.
11.	J. B.	28 yrs.		Pityriasis.		–	–
12.	M. R. H.	1. Female		Anemia.		–	–
13.	M. R. H.	2. "		Gastritis.		–	–
14.	M. R. H.	3. "		Colitis.		–	–
15.	F.			Syphilis-cystitis.		–	–
16.	G. P.	27 yrs.		Hypospadias-cystitis.		–	–
17.	S. Mrs.	22 yrs.	Suspected—husband infected?			–	–
18.	S. baby (daughter).					–	–
19.	H.	30 yrs.		Syphilis.		–	–
20.	G. Mrs.	26 yrs.		"		–	–
21.	P. S.	45 yrs.	Old G. C. hist. Stricture.	Tbc. bladder. (Wass. +)		–	+
22.	E. B. N.	30 yrs.	Old G. C. hist. Clin. cure. ?	Syphilis. Gc. found later.		–	++
23.	J. M.	26 yrs.		Syphilis.		–	–
24.	M. M.			"		–	–
25.	P. Dr.	38 yrs.	Exposure. No discharge.	Gonophobia.		–	–
26.	B. Miss.		2 abortions. Smears.–	Leucorrhea.		–	–
27.	S. G.	27 yrs.	Smears.–	Prostatitis.		–	–
28.	S. Mrs.	?		Syphilis.		–	–
29.	S. C. Mrs.		Smears.–	Leucorrhea; vesical pain.		–	–
30.	F. M.	22 yrs.		Varicocele.		–	–
31.	S. D.			Syphilis.		–	–
32.	R.	45 yrs.	1912 et seq.	Psoriasis.	—8/4.	–	++
33.	B. A. B. H.		?	Chancroid-Bubo.	—8/4.	–	–
34.	R. H. L.	40 yrs.	?	Gout.	— "	–	–
35.	J. K.		None.	Epithelioma.	—	–	–
36.	Case 5. Male.	30 yrs.	"	Asthma.		–	–
37.	S. Mrs.	30 yrs.		Syphilis. Wass.+		–	–
38.	S. child.	5 yrs.		" inherited–		–	–
39.	S. baby.			" " Wass.+		–	–
40.	B. Mrs.	?		General Exam.		–	–
41.	W.	45 yrs.		Alcoholism.		–	–
42.	Ct. G.	38 yrs.	1st 20 yrs. ago. Various attacks.	Wass. +			
				Syphilis, pain in spine.	6/12/15	–	–
	"			Syphilis.	7/27	–	–
43.	P. M.	46 yrs.		"		–	–
44.	D. Dr.	44 yrs.	Old history, no discharge.	" (Wass. ±)		–	++
45.	S. S.	38 yrs.		"		–	–
46.	H. R.	42 yrs.	Denied. Smears.–	Stricture from injury.	6/12.	–	–
	"				7/29		
					6/18		
47.	H. S.	50 yrs.		Syphilis, joint pains,		–	–
	"			"	7/12	–	–
48.	C. E. M.	45 yrs.		" " " "		–	–
49.	E. A. E.	?		"		–	–
50.	R.	?	Only Gc., 1901. Clin. cure.	"		–	–

DISEASES OTHER THAN GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	DISEASE.	DATE.	P.	W.
51.	S. Mrs.	?		Syphilis ?		–	–
52.	T. Dr. J. S.	45 yrs.		"		–	–
53.	R. Mrs.	?		Came for Wass.–		–	–
54.	R. G. L.	29 yrs.		Syphilis.		–	–
55.	J. K. G.	34 yrs.	Denied. Chr. Disch.	Syphilis.		–	–
56.	N. H. W.	40 yrs.		"		–	–
57.	M. M.	34 yrs.		"		–	–
58.	A. S.	40 yrs.	Denied. Chr. Disch. No. Gc. ever seen.	Strictures.		–	–
59.	E. J. B.	38 yrs.	1901, 1903, 1906. stricture, clin. cure.	Syphilis.		–	–
60.	V. V.	35 yrs.	1900, 1906. Lues, 1906.	Tabes.		–	–
61.	M.M.			Came for Wass.–		–	–
62.	D. M. R. H.			Syphilis.		–	–
63.	B. "			"		–	–
64.	L. L.	32 yrs.		" ?		–	–
65.	C. A. M.			"		–	–
66.	A. C. N.	27 yrs.		Tbc. kidney.		–	–
67.	E. S.	27 yrs.	3 or 4 attacks, last 1912. Clin. cure.	Syphilis.		–	–
68.	F. Dr.	40 yrs.		"		–	–
69.	H. Mrs.		Denied.	Syphilis. Wass.+		–	–
70.	C. M.	37 yrs.		"		–	–
71.	C. S. J.	42 yrs.		Congenital "		–	–
72.	E. B.	34 yrs.	Only 1902. Clin. cure.	Hydrocele.		–	–
73.	T. H.		Denied.	Came for Wass.–		–	++
74.	J. S.	61 yrs.	"	Papilloma of cheek, probably on luetic base.			
				Wass.+	7/19	–	++
	"				7/30	–	–
75.	G. R.			Syphilis.		–	–
76.	E. M.	22 yrs.		Varicocele.		–	–
77.	L. Miss			Tumor of breast.		–	–
78.	B. H.	30 yrs.		Syphilis.		–	–
79.	A. E.	29 yrs.		"		–	–
80.	P.	?		Came for exam.		–	–
81.	H. L.	36 yrs.	1898, 1909. Clin. cure.	Impotence.		–	–
82.	H. K.	25 yrs.	Denied. Intermit. Disch.	Stricture.		–	–
83.	W. Mrs.		" " "	Syphilis (Wass.–).		–	–
84.	A. G.		Only 1914. Clin. cure.	"		–	–
85.	L. K.	30 yrs.	Only 1912. Clin. cure.	"		–	–
86.	G. B.	28 yrs.		"		–	–
87.	J. H.	24 yrs.		"		–	–
88.	Hosp. case.		Doubtful, supposed –	?	5/25	+	+
89.	A. F.	58 yrs.	No. Gc. history.	Tumor of Bladder.	5/28	–	–
90.	R. Z.	40 yrs.	"	Came for examination.		–	–
					5/10		
91.	H. M. S.	?	"	Bladder operation.		–	–
92.	J. C. C.	47 yrs.	Gc. 1895.	Post-urethritis. Smears–; bladder tumor.		–	+
93.	Mrs. M.	?	No. Gc. history.	Fear after exposure. Smears.–		– –	– –

DISEASES OTHER THAN GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	DISEASE.	DATE.	P.	W.
94.	R. C.		No. Gc. history.	Alcoholism.		–	–
95.	Dr. M.		"	Came for examination. Lues.		–	–
96.	M. R. H.		"	"		–	–
97.	C. Mrs.	30 yrs.	"	Acne rosaceae.		–	–
98.	A. D.		Obscure.	Rheumatism.	4/22	++	++
99.	A. D.	30 yrs.		Clear urine, shreds.–	7/26	–	–
100.	W. H. R.	43 yrs.	1890 (lues, 1895)	Bright's disease.		–	–
101.	J. W. F.	41 yrs.	No. Gc. history.	Syphilis.		–	–
102.	Dr. T.	40 yrs.	Uncertain.	Came for exam. and Wass.		–	–
103.	V. L. W.	43 yrs.	?	Syphilis.	– 8/5	–	–
104.	R. B.	46 yrs.	?	Hernia.	– 8/5	–	–
105.	S.	41 yrs.	?	Burn.	– "	–	–
106.	J. F.		?	Syphilis.	– "	–	–
107.	W. J.		?	"	– "	–	–
108.	P. D.	33 yrs.	?	"	– "	–	–
109.	J. K.	40 yrs.	?	Impotence.	– "	–	–
110.	W. H. R.	42 yrs.	?	Dyspepsia	– "	–	–

A review of Table I shows that the commercial antigen gave positive reactions in 5.4 per cent of unsuspected cases, and the W. antigen in 13.6 per cent. A majority of these positive cases gave histories of gonorrhea, and in the others it was impossible wholly to exclude a past infection. An illustration is found in Case 42. A barber denied gonorrhea at the time the test was made and later admitted various attacks, stating that his present condition gave him no trouble. Six weeks later the gonococcus test had become negative.

There is no relation between the gonococcic and the syphilitic antigens. In the table are 57 cases of syphilis. A majority gave positive Wassermann reactions, and negative gonococcus reactions. In 7 cases the W. antigen gave positive reactions, the P., 4. In these the Wassermann reaction was positive in 4, doubtful in 2, and negative in 1.

TABLE II.

ACUTE GONORRHEA.

(To the Fifth Week.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	GC. PRESENT.	DATE.	P.	W.
1.	J. L. P.	40	1895, April, 1915.	+	5/29/15	+	++
2.	W. P. G.	30	1910, 5/15/15.	+	5/28	–	–
3.	M. C. C.	36	1900, 1909. 5/7/15.	+	5/12	–	++
4.	H. B.	23	1914. 5/7/15.	+	5/7	–	–
	"	"	"	+	5/13	+	++
5.	A. M.	19	4/10/15.	+	5/10	+	++
	"			–	7/20	+	++
6.	H. B. S.	30	4/16/15. No Gc. after 4/19.	–	5/1	–	+
7.	M. W.	32	1908, 1913. 4/15/15.	+	4/26	–	++
	"			+	5/11	–	++
	"			–	6/2	±	±

ACUTE GONORRHEA. (Continued.)
(To the Fifth Week.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	GC. PRESENT.	DATE.	P.	W.
8.	Van D.	20	4/10/15. Marked cystitis.	+	4/26	++	++
9.	J. R.	69	4/15/15. Marked edema.	+	4/21	–	++
10.	P. B.	18	1914. 4/28/15.	+	4/28	–	++
11.	S. A. H.	27	1913. 5/5/15.	+	5/8	+	++
12.	St. C.	22	3 weeks' duration.	+		–	+
13.	L. K.	20	" "	+		–	+
14.	J. L.	20	2 " "	+		–	–
15.	A. F.	21	3 " "	+		–	–
16.	Case 2.		4 " "	+		–	++
17.	H. V. D.	24	1909 (lues, 1912) 5/4/15.	+	5/10	–	–
	"		" " "	?	6/4	++	++
18.	S. G. C.	35	1901, 1903, 1910. 6/5/15.	+	6/8	–	–
	"			+	6/20	–	–
	"			?	7/23	–	–
19.	L. C.		1906, 1909, 1911, 1914, 2 weeks' duration.	+		±	++
20.	T. J. B.	35	1910, 1914. 6/9/15.	+	6/15	–	–
	"			+	7/15	+	+
	"			+	7/19	–	+
21.	A. C.	28	1911, 1912. 7/9/15.	+	7/16	–	–
22.	H ?		1910. 7/15/15.	+	7/17	–	–
	"			+	7/26	+	++
23.	R. B.	40	7/20/15. (Lues. 4 yrs.)	+	"	+	++
24.	H. A.	25	1911. 4/26/15.	+	4/26	–	+
25.	A. G.	40	5/3/15. Vaccine.	+	5/5	–	+
26.	H. B.	45	? 1908. 7/25/15. Disch.	+	7/27	–	–
	"		Rheumatism,	+	8/13	++	++
27.	G. C.	25	1913. 1914, 1915. 7/22/15. Disch.		8/13	–	–
28.	A. C.	28	1908, 1909, 1910, 1914. 7/25/15. Disch. vaccine.		7/27	–	±
29	C. B.	34	1913, 1914, to 5/11/15; 7/30/15. Disch. vaccine.		7/30	–	–
30.	J. L. D.	22	8/1/15. " "		8/5	±	–
31.	M.	"	1911. 7/25/15. "		8/5	–	–
32.	A. B. H. E. P.		? Epidid.	–	8/6	+	++
33.	" 9.		? "	–	"	–	–
34.	" 10.		Three weeks. "		"	–	±
35.	Sku.	27	1912, 1913. 8/9/15. Disch. Vaccine.	–	8/10	–	–
36.	A. B. H.	23	Two weeks. Epidid. Very acute.		8/13	–	++

The sera of the acute cases reacted with the commercial antigen 14 times, or 40 per cent. Of this number, only 2 cases were ++. The earliest reaction appeared on the fourth day, and three cases reacted within the first week. These figures show a higher percentage of positive reactions usually obtained with watery antigens within the first month of the disease. The W. antigen gave positive reactions in 26 cases, or 72 per cent. Of these, 18 were ++. Three occurred on the second day of the infection, and 8 within the first week.

Early reactions did not appear to have any relation to previous attacks of gonorrhea, as they occurred in primary infections, and were delayed in some cases that acknowledged many previous attacks. We have the impression that

those cases showing the greatest amounts of local reaction to the infection give the earliest positive reactions. The test was repeated in some cases after the expiration of the fourth week, but the results are included in this table.

TABLE III.

SUBACUTE CASES OF GONORRHEA.

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	GC. PRESENT.	DATE.	P.	W.
1.	Dr. O., 1.	26	6 weeks' duration. Epididymitis	+		−	+
2.	" 2.	28	5 weeks' duration. Epididymitis.	+		−	−
3.	A. B. H. 4.		Doubtful. Epididymitis.	−		−	−
4.	M. E. C.	20	2/1/15.	+	5/22	−	+
5.	Bal.	21	1913. 2/1/15.	+	5/12	−	−
6.	Temp.	20	1914. 2/1/15.	+	5/8	−	++
7.	H. H. H.	22	3/15/15? Rheum., five days later. Eye? No disch.	−	4/24	−	−
	"			−	5/5	−	−
	"			−	5/14	−	−
8.	C. H. McC.	25	3/4/15.	−	5/5	−	+
9.	A. L. M.	21	1913. 3/4/15. No. Gc. after 3/29.	−	4/3	−	−
10.	F. S. M.	30	2/20/15. Double epidid.	−	4/29	−	−
				−	5/6	−	−
11.	A. J. F.	28	3/5/15. Lame heel.	−	2/29	−	++
12.	B. L.	21	7 weeks' duration.	+	6/12	+	++
	"			?	7/19	+	+
13.	Dr. P.	30	1914. 6/7/15.	+	7/24	++	++
14.	F. A. E.	26	6/1/15.	+	7/15	++	++
15.	K. H.	23	1910, 1912. 3/1/15.	+	"	+	+
16.	A. P.	25	1911. 5/10 prost.	+	7/24	−	+
17.	Miss C.	20	? Bartholinitis, cervicitis.	+		−	+
18.	A. B.	26	5/15/15. Disch.	+	5/7	−	−
19.	Case B.		4/1/15. Clin. cure.	−	7/27	−	−
20.	G. C. R.		6/18/15. Disch, slight.	−	8/6	−	−

Of the 20 cases tested in the subacute stage, 4 responded positively to the commercial antigen, 11 to the fatty antigen, or 23 per cent and 64 per cent respectively. We observed in the cases noted in this and the following tables a considerable variability in the occurrence of positive reactions, a point of importance in connection with the low percentage. It has been observed by others,[1] and repeatedly noted in our own work, that alternating positive and negative reactions occur during the progress of an attack of gonorrhea, which appear to indicate phases of the infection. The precise significance of the fluctuations is not understood, but they appear to occur oftenest during the period designated as subacute. It is, therefore, not infrequent that a considerable series of cases will give negative reactions at one time, and positive reactions at another. It has seemed to us that the negative reactions were associated with lessened or absent discharge, and disappearance of gonococci.

Case 7 illustrates the value of repeatedly negative reactions. Early in the illness a slight discharge was noted, but no gonococci were ever found. The

[1]Irons and Nickels: Jour. Infect. Dis., April, 1915.

case was pronounced to be gonorrheal rheumatism and iritis, but the serum reactions cast doubt on the diagnosis. The patient recovered promptly under salicylates.

TABLE IV.

CHRONIC GONORRHEA.

(Four Months to One Year.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	CONDITION.	GC.	PRESENT.	DATE.	P.	W.
1.	D. A.	25	12/1/14.	Stricture.		−	5/28	+	+
2.	K. H.	23	1910, 1912.	Ac. prostatitis.		+	5/26	−	−
	"		3/1/15.			+	7/19	+	+
3.	E. H. S.	37	1900. 12/26/14.	Discharge.		−	5/25	−	−
4.	A. B. H. 6.	?	Indefinite.	Smear.		?	"	−	−
5.	J. K.	?	8 months.	Strict.		−	5/7	−	−
6.	L. A. N.	34	12/14/14.	Disch.		+	5/12	−	−
7.	A. G. S.	23	Oct., 1914. Recur., 2/1/15.	Stricture.		+	5/12	±	+
8.	A. K. F.	40	Dec., 1914.	Disch. (Lues)		+	5/6	−	±
9.	J. R.	?	1/25/15.	" "		+	5/6	+	++
10.	J. B.	?	Indefinite.	Prostate abscess.		Rectal ulcer.	4/29	−	−
11.	A. T.	21	6/1/1914. Recur., Dec., 1914.	Stricture.		−	5/8	−	++
12.	Case 1.	?	4 months.	Disch.		+	3/1	−	++
13.	Case 3.		4 months. −	"		?	"	−	++
14.	J. B. R.	22	Only 10/1/14.	" A. M.		+	6/4	−	−
	"			"		−	6/4	−	+
	"			"		−	7/19	−	+
	"			"		−	7/26	−	−
15.	L. A. P.	25	4 previous attacks, 2/1/15.	" A. M.		−	6/5	−	+
16.	H., Miss.	?	Indefinite.	Cystoscopy.		Ureteral Catheters.	6/5	+	++
	"						7/31	−	−
17.	X. M.	30	5 months healed epidid. No disch.			−	7/29	−	−
18.	L. V.	28	Only 7/1/14. No discharge since 4/1/15.			−	7/1	−	−
19.	A. U.	25	Months.	Threads only.		−	7/1	−	−
20.	O.	30	Indefinite, chronic.	Disch.		+	6/5	−	++
21.	A. B. H. 423-7.		" "	No disch.		−	"	−	−
22.	R. O.	30	" Gc. found once.	" "		−	5/13	−	−
23.	A. W.	?	Two months.	Disch.		+	5/13	++	++
24.	G.	"	" chronic.	No disch.		−	4/26	−	−
25.	W.	"	" "	" "		−	"	−	−
26.	A. M.	"	Five months, chronic.	Disch.		−	7/26	−	−
27.	C. H.	"	Six "	" A. M.		−	"	−	−
28.	O.	31	11/1/14. Rheum., 12/14.	No disch.		−	"	−	−
29.	W.	30	Doubtful.	" " Threads.		−	"	−	−
30.	McN.			Disch.		+	"	−	+
31.	L. C. A.	26	1912. 1/1/15.	Strict. A. M. disch.		−	8/12	−	−

CHRONIC GONORRHEA (Continued.)
(Four Months to One Year.)

NO.	NAME.	AGE.	HISTORY OF GONORRHEA.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
32.	E. W. L.	24	1910. 3/1/15.	Epidid. Clin. cure. Furnucle.	–	8/9	–	++
33.	Shel.	"	1914. et seq.	A. M. disch.	–	"	–	
34.	Kr.	30	Months.	"	–	8/13	–	+
35.	Hil.		1/1/14 et seq.	"	–	"	–	+

Thirty-five cases examined within the time limits of the table, but only 11 of which showed definite presence of gonococci, gave positive reactions with the commercial antigen in 6 or 17 per cent, and with the W. antigen in 17 or 48 per cent. It will be apparent that if the cases in this division had been limited to such as showed signs of remaining infection, in other words, omitting those free from discharge or other symptoms, the percentage of positive reactions would have been much higher. Number 14 well illustrates a periodical fluctuation in the course of a chronic case.

TABLE V.

LONG-STANDING AND REPEATED ATTACKS OF GONORRHEA.

NO.	NAME.	AGE.	HISTORY.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
1.	Dr. J.	44	Only 23 yrs. ago.	Sac. Iliac pain. Disch. slight.	–	5/15	++	++
2.	E. O.	28	1909, 1912, 1913, 1914, 1915.	Chronic epid. No disch.	–	"	–	–
	"			After exercise, disch.	–	7/12	+	+
3.	M. A.	40	1902, 1914. Rheum.	Well.		5/15	–	–
4.	A. B. H. 7		Indefinite.	4th epididy.	–	5/25	–	+
	" (Gitas)				+		+	++
5.	" 8		Old and indefinite-luetic.	No disch.	?	5/24	–	–
6.	J. S.		" " "	Disch.	?	5/24	+	+
7.	B.		1913.	Chr. post.	–	7/27	–	–
8.	M. A. R. C.		Old and indefinite-luetic.	Disch.	?	"	++	±
9.	D. R.		" " "	"	?	"	++	++
10.	J. J. L.	49	Continuous for 20 yrs.	"	+	4/15	–	++
	"		After vaccine,	No. disch.	–	5/13	–	–
	"		" "	" "	–	5/20	–	–
11.	B. T. H.	51	4th attack in 14 yrs.	Strict. Disch.	?	5/25	++	++
12.	H. A. L.	29	1913, 1914. 4/21/15.	Disch., A. M.	–	5/20	–	++
13.	H. B. L.	36	1905, 1912, 1915.	"	–	5/20	–	++
14.	J. C	?	1905, 1906.	Disch., A. M.	–	5/10	–	+
15.	P. H.	?	1903, 1909, 1914.	" "	–	5/14	–	+
16.	J. G. O.	?	1900 only.	(Lues) Disch. A. M	–	"	–	+
17.	L. K.	32	Only 1905.	" "	–	"	–	+
18.	G. D.	43	1909.	Strict. Disch. Epid.	–	4/1	–	–

LONG-STANDING AND REPEATED ATTACKS OF GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
19.	T. R.	65	1870.	Strict., 1885. Disch. A. M. Cloudy urine.	−	5/12	−	+
20.	W. J. S.	45	1895. 1904. Recurrences.	Disch.	−	4/22	++	++
	"			" epididy.		5/10	−	++
	"			No disch.	−	7/15	−	−
	"				−	8/10	+	+
21.	J. C.	31	3 attacks.	Strict. prostatic. Disch.	?	5/8	−	+
22.	H. B.	28	1903, 1909.	" "	−	5/7	−	−
23.	A. L.	25	1911 intermittent.	" Disch.	−	5/6	−	+
24.	A. T.	39	4 attacks in 8 years.	"	−	5/6	−	−
25.	F. P. W.	44	6 to 8 attacks.	Strict. multiple. Disch.	−	5/6	+	++
26.	H. C.	32	1903, 1911.	Infiltrated urethra. Disch.	−	"	+	+
27.	H. I. A.	?	Old history.	No signs.		"	−	−
28.	H. D.	44	1892, 1897. Smeared 4/3/15.	After vaccine.	−	5/5	−	−
29.	F. P.	30	1911, 1913, 1915.	Strict.	−	"	−	−
	"			"	−	5/20	−	−
30.	P. R.	40	1904, 1909, 1914.	Strict. 1914, No signs.	−	5/5	−	−
31.	J. L.	44	Intermittent for 24 yrs.	Disch.	−	5/25	+	++
	"			"	−	5/20	++	++
32.	A. P.	30	2/1/13.	No signs, vague pains.	−	4/21	−	−
33.	C. L.	30	Rheum. 2½ yrs. ago.	Constant disch.	?	4/21	+	++
	"			" "	?	"	−	++
34.	F. A.	40	1901. Long use of sounds.	No disch.	−	"	−	−
35.	A. D.	35	1902, 1903, 1914.	Disch.	+	4/28	−	−
	"			No disch., urine clear.	−	6/8	−	−
	"			After epidid. clin. cure.	−	8/4	−	++
36.	H. C. C.	33	3 attacks to 1913.	Iritis, 2/26/14. Strict., 12/31/14. (P +). No Gc. ever found.		4/26	−	−
37.	C. W.	24	1908, 1914, 2/12/15.	Constant disch., prostatitis.	+	4/26	−	++
38.	G. K.	?	Old history strict.	No signs, Wishes to marry.	−	"	−	−
39.	A. H.	25	1911	"	−	"	−	−
40.	O. A. P.	30	1907, 1912. (Lues, 1910)	Prostatitis. Disch.	?	"	+	+
	"				−	7/12	−	−
41.	Z. C.	40	1901, 1905.	Intermit. disch.	−	5/10	−	−
42.	Case 4.	?	4 years' duration.	" "	+	3/1	++	++
43.	" 6.	?	Long recurring.	" "	+	"	+	+
44.	J. D. H.	28	1906 (psoriasis, 1911).	Disch. 10 days, prostatitis.	?	6/3	−	++

LONG-STANDING AND REPEATED ATTACKS OF GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
45.	A. R. F.	43	Only 1901.	Disch. A. M. Pain in back.	–	6/4	–	++
46.	A. H.	34	Only 1901.	Disch. A. M. Wishes to marry.	–	6/4	–	+
	"					7/29	–	±
47.	A. S.	27	Only 1912. (Lues, 1908)	Marriage? No disch. Specks. Pain in shoulder.	–	6/8	–	++
48.	J. S.	44	1896, 1902.	Scrotal dermatitis.	–	6/4	–	–
49.	D. M. S.	30	Only 1911.	No signs. Wants children.	–	"	–	–
50.	B. H. N.	32	1912, 1914.	Intermit. disch. present.	–	6/9	–	+
				No disch.	–	7/15	–	–
51.	J. J. A.	39	1901, 1903, 1906.	Clinical cure.	–	6/9	–	–
52.	F. H. S.	30	1911. Seborrhea.	Wishes to marry. No signs.	–	6/12	–	–
53.	L. M. B.	59	Many attacks, 25 years ago.	Glycosuria. Prost. Disch.	–	"	–	++
54.	I. L.	50	1895. Many attacks (lues).	Disch.	–	6/15	++	++
	"			" ?	–	7/21	–	–
55.	E. W.	56	Denies Gc. Has urethritis	Prostatitis. Discharge.	–	6/12	–	–
	"			No disch.	–	7/19	–	–
56.	A. W.	38	4 or 5 attacks, last 1910.	Disch.	–	6/16	++	++
	"				–	7/19	–	–
57.	G. F. D.	33	1903.	No signs sterile. ?	–	6/16	–	–
58.	Mrs. Z.	?	Gc. and lues, 1912.	No signs, sterile, ?	–	6/16	–	–
59.	H. F.	26	Test + 1912 lues.	Joint pains. No discharge.	–	"	–	–
60.	H. J.	26	1905, 1907, 1911, 1914.	No discharge. Pains in joints.	–	"	–	+
61.	J. N. W.	33	1900, 1904, 1909.	Recur. Vesiculitis. Stricture. Discharge.	?	6/12	–	++
62.	E. K.	28	1906, 1908, 1910, 1914.	Prostatitis. Discharge.	–		–	–
63.	H. L.	35	1903, 1914.	Intermit. disch. A. M.	–	7/15	–	+
64.	F. D. N.	51	1895, 1911. Irregular disch.	No disch.	–	"	–	–
65.	L. P.	36	1904, 1914.	Continuous disch.	–	7/17	–	–
66.	K. M.	32	1910.	No signs. Marriage?	–	7/15	–	–
67.	H. L.	40	1910.	Disch.	+	"	–	–
68.	A. R. W.	28	1909.	Pain in joints and kidney. No discharge.	–	7/19	–	–
69.	B. S.	37	1912 (Test+)	Slight disch.	–	7/24	–	–
70.	Dr. D. S.	?	Only 1904. Comp. cured. 1907.	Perineal pain. Disch pus. Diph.	–	"	–	–
71.	B. W.	48	2 attacks, last 20 years ago.	Enl. prost. Disch.	–	7/26	–	–
72.	J. L.	40	1893, 1900.	Impotence. Threads, Occasional disch.	–	7/26	–	–
73.	M. J. K.		1912, Comp.	Drinking. Disch. A.	–	"	–	–

LONG-STANDING AND REPEATED ATTACKS OF GONORRHEA. (Continued.)

NO.	NAME.	AGE.	HISTORY.	CONDITION.	GC. PRESENT.	DATE.	P.	W.
74.	L. C.	50	1906.	Disch. 4 weeks ago. No disch.	–	5/10	–	–
75.	F. S.	58	1910.	Prostatitis. Discharge. Staphylococci.	–	4/29	–	–
76.	E. K.	26	1910. Recurrences.	No disch. Vesiculitis?	–	7/29	–	–
77.	W. Y. D.	30	1912.	Stricture, Specks, Smears. Retention.	–	6/12	–	–
	"				–	7/29	–	–
78.	E. S.		Indefinite.	Constant discharge.	+	8/5	–	++
79.	A. P.		1910 et seq.	Epidid. Orchitis. Disch.	–	8/13	+	++
80.	J. J. D.		1897.	Epididy. Vasitis. Sterile.	–	8/9	–	–

Eighty sera from cases of long-standing gonorrhea with repeated attacks gave 19 positive reactions with the commercial antigen, or 24 per cent, and 40 positive reactions with the W. antigen, or 50 per cent. Of these patients, 12 were clinically cured, 52 showed a discharge, and in only 10 were gonococci to be found. It is in this class that complement fixation is of the highest value. The percentage of positive reactions would be much higher, 37 per cent with the commercial antigen and 80 per cent with the fatty antigen, if the cases clinically cured and those free from discharge had been eliminated.

TABLE VI.

GONORRHEA IN CHILDREN (FEMALE).

(Unsatisfactory Histories.)

NO.	NAME.	AGE.	DURATION.	GC. PRESENT.	REMARKS.	DATE.	P.	W.
1.	S.	5	8 months.	+			–	++
2.	W.	3½	1 year.	–			–	–
3.	R.	8	"	–			–	–
4.	R.	3	3 months.	+			–	++
	"		4 "	?			–	++
5.	K.	8 mos.	3 months.	+			–	++
	"		4 "	+			–	++
6.	G.	5	4 months.	+			–	++
	"		5 months.	+			–	++
7.	H.	13 mos.	6 weeks.	+			++	++
	"		10 "	+			+	++
8.	P.	1 year.	3 months.	+			–	++
9.	F.	7 years.	1 year.	–	Diptheroids. Gc. never found.		–	–
	"		13 months.	–	" "		–	–
10.	E.	10 mos.	2 months.	–	" "		–	–
	"		3 months.	–	" " (Faucial Diphtheria).		–	–
11.	D.	2 years.	1 month.	–			–	–
	"		2 months.	–			–	–
12.	G.	15 mos.	8 months.	+			–	+
	"		9 months.	–			–	±

GONORRHEA IN CHILDREN (FEMALE.) (Continued.)
(Unsatisfactory Histories.)

NO.	NAME.	AGE.	DURATION.	GC. PRESENT.	REMARKS.	DATE.	P.	W.
13.	E. F.	7 years.	Years.	–	Gc. never found.		–	–
14.	R.	10 years.	?	+		5/4/15	++	++
	"			+		5/19	–	++
	"			+		5/28	–	++
	"			+	Vaccine.	6/30	–	–
15.	F. M.	5 years.	Months.	–			–	–
16.	I. C.	4 years.	"	–			–	–
17.	E. O. N. N.	8 years.	"	+			–	±
18.	D. P.	12	Long-standing.	+			–	+
		"	?	+			–	–
		13	1 month.	+			–	+
20.	F. Mas.	14	?	–			–	–
21.	L. C.	10	Months.	+			–	–
22.	T. S.	12	"	+			–	–
23.	M. W.	6	"	+			++	++
	"		1 month.	+			–	++
24.	A. T.	9	Months.	+			–	–
25.	S.	12 years.	Long-standing. vaginitis.	+		5/29	–	++
	"					6/2	–	++
26.	P. M.	5	Long-standing. vaginitis.	+		5/6	–	++
27.	C. D.	12	5 weeks ? vaginitis	+		"	–	++
28.	N. B.	8	4 weeks vaginitis	+		"	–	++
29.	M.	4	?	–		"	–	–
30.	O. F.	2	1 yr. Vaccine.	+		4/24	–	+
	"		Vaccine.	–		5/7	–	–
31.	S. I.	5	Doubtful.	–		4/29	–	–
32.	B. F.	8	Doubtful.	–		"	–	–
			Long-standing.			"	–	+
33.	R. C.	6	Vaginitis.	+				

Thirty-three female children, a majority with unsatisfactory histories, showed positive reactions to the commercial antigen in only 3 instances, less than 10 per cent, and to the W. antigen in 18, 54 per cent. We have been unable to account for the very low percentage in the former, unless the explanation may be found in the antigen. The W. antigen was made from various strains of gonococcus, 7 of which were isolated by one of us from cases of specific vaginitis in girl children, whereas we understand that few, if any, of the strains used in making the commercial antigen were obtained from such source.[1]

Table VII shows the number of positive reactions obtained with both antigens in cases where gonococci were demonstrable. There is also shown the number of positive reactions where gonococci were not found. Certain apparent discrepancies in totals are explained by the fact that in some instances where the test was repeated on the same serum the gonococcus was present on one occasion and absent on another.

[1]The vaginitis strains referred to have only recently been presented to the laboratory of Parke, Davis and Company.

TABLE VII.

	Gc. present.	P.	W.	Gc. absent.	P.	W.
Acute	32 cases	12	24	7 cases	1	6
Subacute	13 cases	4	9	9 cases	1	3
Chronic	11 cases	4	8	22 cases	2	8
Long-standing	11 cases	4	8	60 cases	13	30
Children	20 cases	4	18	16 cases	0	3

Analysis of the above shows, on the whole, what appears to be a lower percentage of positive reactions with the commercial antigen than has been obtained and reported by other workers. There are several factors to be considered in this connection. By far the greater number of cases, in fact nearly all, except the children, who were hospital cases were private patients, and received the best of care. The complicated cases, usually neglected, and ultimately found in dispensaries and hospitals, are few. Among the latter the proportion of positive reactions is much higher. Again, tabulation of cases of the class dealt with according to the age and duration of the infection, some of the cases appearing clinically cured, must necessarily yield a lower proportion of positive reactions than a series in which all but the doubtful cases have been excluded. A third factor is the variability or fluctuation of reactions that occur during the course of an infection. It has been observed in this work, and by other writers, that alternation of positive and negative reactions occur frequently during the continuance of gonorrhea. The reason for this is not explained, although it is unquestionable that exercise, alcohol and sexual excitement have decided influence. The administration of antigen in form of ordinary vaccine has no influence whatever. With these facts in mind, it is not difficult to understand how one series of cases may show more positive reactions than another.

There is no question but that a single negative reaction in a suspected case has no value, whereas repeated negative reactions in the absence of clinical signs of gonorrhea are of great value. We strongly urge repeated tests in doubtful cases giving negative reactions. On the other hand, a single positive reaction, even in the absence of clinical signs, is of the greatest importance and indicates sensitization by gonococci. Our experience has shown that stimulation in such cases, with sounds and silver, frequently is followed by the appearance of cocci in the exudate.

The effect of epididymitis upon the complement fixation has not been marked in this series or in our previous work, and we believe this phase of the disease to have no influence in producing positive reactions, but rather the contrary. We have observed repeatedly negative reactions throughout the course of epididymitis, and have observed positive reactions disappear with an oncoming attack. The severity of the complication has appeared to be associated with negative reactions, mild cases giving a much higher proportion of positives. Theoretically, one would expect such relationship in view of the well-known clinical phenomenon of disappearance of exudate and cocci from the urethra during an inflammation of the epididymis, a few of the severest cases of which unquestionably induce cures of the urethral infection.

Comparison of the two antigens shows the superiority of the Warden antigen over the best of the watery antigens. Where the latter gave positive re-

actions, the former invariably gave positives, and, as a rule, to a greater degree, save in one instance only. With the latter also there is much less fluctuation. The positive reactions appeared earlier, persisted longer, and occurred in a larger number of doubtful cases. We believe this antigen can be still further improved. The data in this work appear to us to warrant the following conclusions:

1. An alcoholic solution of the fats of the gonococcus serves as antigen in the gonococcus complement fixation test, and is superior for the purpose to the watery antigen of commerce.

2. We concur in the opinion that positive reactions are always of value. Repeated negative reactions in the absence of clinical signs are of great value. A single negative reaction has no significance whatever.

3. While the sera of normal persons have been thus far wholly negative, and while it is admitted that positive reactions are largely confined to cases where the gonococcus is, or has recently been, present, nevertheless, the evidence, as a whole, leads us to believe that a positive reaction indicates the presence in the serum of some substance which reacts with the antigen (antibody?) to produce fixation of complement, and not necessarily the presence of a focus of gonococcus in the body.

We wish to thank Dr. E. W. White and Mr. Richard Zickman for much assistance in the preparation of this paper.

FETAL ERYTHROBLASTOSIS: FETAL ERYTHROBLASTOMATOSIS *

By Paul G. Woolley, M.D., Cincinnati, Ohio.

CONGENITAL general edema is a subject which has been engaging more and more attention. About it a very considerable literature has been developing, not because the cases which exhibit the condition are very frequent, for they are not, but because of the opportunities for theoretic perambulations. Ballantyne, Chiari says, was able to find but 70 cases in the literature between 1614 and 1898. Recently, especially since the awakening of interest by the communications of Schridde, the reports are becoming more frequent.

In its characteristic form, congenital general edema is evident at birth, and appears to have developed during the period of the fetus in the uterus. Because of the swelling of the tissue, delivery is difficult, and after the expulsion or forcible removal of the infant, the cord and placenta are, like the child itself, found to be waterlogged.

Concerning the causes of the condition, something is known of some cases; nothing, of others. They are grouped as follows:

1. Hydrops ascites congenitus caused by disturbances in the portal circulation; fetal peritonitis; absence of the ductus venosus Arantii; and in certain anomalies of the intestinal tract.

*From the Mary M. Emery Laboratory of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.

2. In a certain group of cases there are anomalies of the heart, of the vessels, or of the uropoietic system of the fetus.

3. In a certain group of cases the edema of the fetus seems to be associated with maternal disease—chiefly nephritis.

4. In another group none of the above mentioned factors are present, and in this it has been supposed that some metabolic anomaly of the fetus develops which finds expression in abnormal blood production and edema.

It is this last group which is most interesting, for in it belong the cases of what has been called fetal erythroblastosis (Raubmann). This very peculiar and almost generally unrecognized complex is characterized, as Schridde said, by the following features:

1. The fetuses (premature as a rule) show universal edema in the form of anasarca, and hydrops of the body cavities.

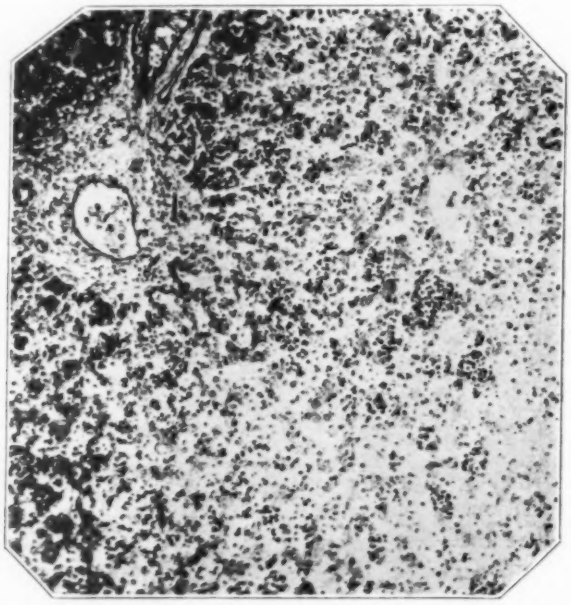

Fig. 1.—A low power photomicrograph of a liver section to show the islands of (chiefly) erythroblasts and the atrophy of the hepatic parenchyma.

2. Most of them show also edema of the placenta and cord.

3. The liver and spleen are markedly enlarged.

4. Microscopic examination of the liver and spleen show unusually large numbers of erythroblasts with large numbers of other myeloid cells, inside and outside the vessels. Also, the liver cells are atrophic, and in the spleen the follicles are absent. In other organs, especially in the kidneys and lymph nodes extramedullary erythroblastic nodules appear, and the blood shows enormous numbers of erythroblasts overshadowing all other elements. Mitotic and rhectic figures appear, and the liver cells and pulp cells of the spleen are colored with iron-containing pigment.

These characters were shown in their entirety by a case sent me by Dr. H. L. Woodward, which, however, was more than usually interesting in that

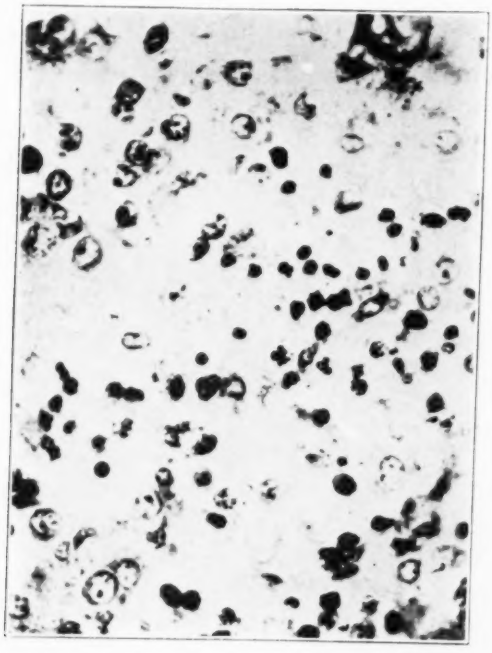

Fig. 2.—An area in a section from the liver showing the edematous and atrophic liver cells, and also numbers of erythroblasts, some of them showing mitosis.

Fig. 3.—Same as Fig. 2, but somewhat greater magnification. The parenchymatous changes are well shown.

the anasarcous fetus was one of a pair of twins. The other twin was apparently normal. Delivery was not forcible, but the delay caused by the delivery of the anasarcous infant led to the death of the healthy one. Both were premature.

Schridde believed that this fetal disease was the result of a severe fetal anemia which was associated with a high grade of reparatory erythropoiesis. The anemia, it was assumed, was of toxic origin. Schridde did not believe it

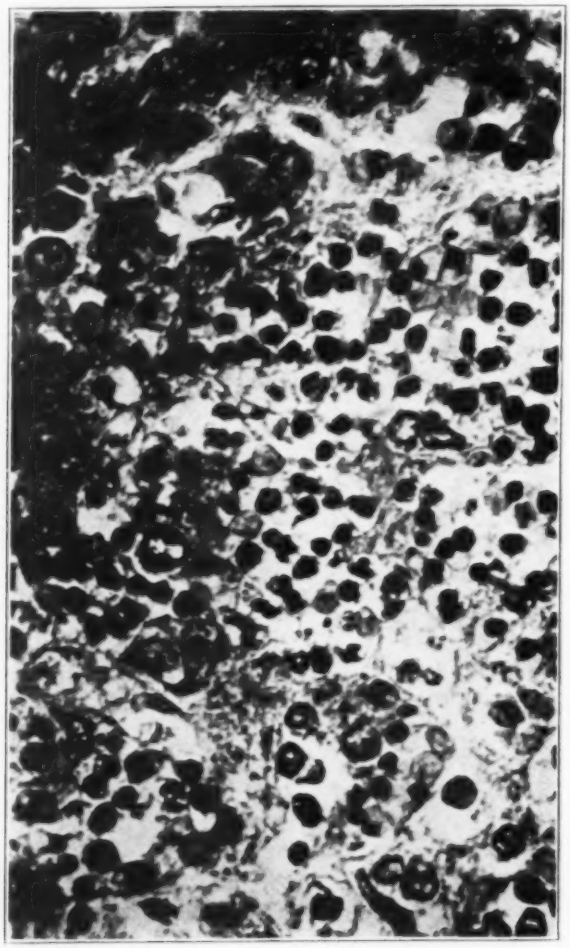

Fig. 4.—Same as Fig. 3, but with still greater magnification.

to be luetic. Fischer called attention to the similarity of the picture to that of *anemia pseudoleukaemia infantum* and suggested that this kakophoniously named disease might be an exaggerated form, or a further development, of fetal anemia in which the criteria might be similar to, though less marked than those of the erythroblastosis type. One of Schridde's cases was derived from a nephritic mother but in this instance he believed that the fetal condition was coincidental and not the result of the maternal condition. This is interesting because there is the tendency to lay these cases at the door of maternal disorders

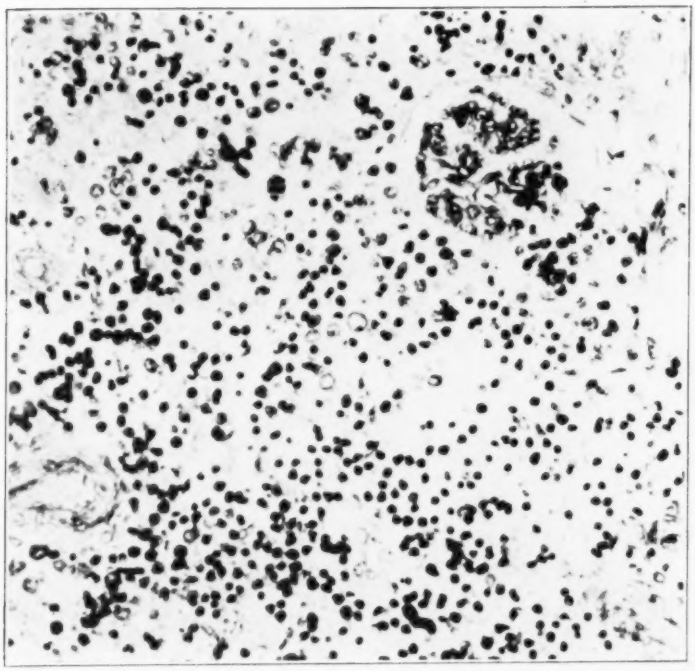

Fig. 5.—A low power photomicrograph of an area in the kidney showing the massing of erythroblasts.

Fig. 6.—Same as Fig. 5, but with greater magnification.

Fig. 7.—Nucleated red cells in the umbilical vein.

Fig. 8.—Same as Fig. 7, but with greater magnification. One erythroblast shows evidence of division.

(Fischer, Wolff). It is generally supposed that the disease is of toxic origin and that the toxic materials, while causing destruction of erythrocytes, at the same time stimulate the blood forming tissues. The origin of the poisons is not known. Certainly they do not come from the maternal blood—or almost certainly. In the case here reported, one twin was healthy.

Schridde and Chiari both incline to the opinion that the condition rests upon some fundamental disturbance of the metabolism of the fetus, though what the cause is they do not know. The question remains whether there is a chronic irritation of the erythroblastic tissues, or whether the primary thing is a destruction of circulating red cells with reparatory overproduction, or both.

It would appear that the anasarca is the expression of the anemia which

Fig. 9.—A placental villus with nucleated red cells in its vessel.

in turn is due to disturbed production and perhaps also to increased destruction, of erythrocytes. The pigmentation of the organs speaks for such destruction. As is very well known, edema is a constant occurrence in all grave anemias, because either there are not enough red cells to carry oxygen to the tissues, or because the hemoglobin content of the cells is not sufficient to attend to the oxygen needs. In either case, the oxidations of the tissues are necessarily slowed and metabolites which normally would be broken down and carried away remain in the tissues, and, because these metabolites are in the nature of acids, they lead to water absorption by the tissues. The anasarca is therefore the expression of a severe grade of anemia. When anatomic defects occur in the body, anasarca also occurs because the blood, capable though it

may be, is not properly oxygenated for obvious reasons. In typical erythroblastosis, there are no anatomic anomalies.

Why the anemia occurs is a more difficult question. There seem to be two possibilities. There may be some chemical irritation of the hematopoietic apparatus which, while it causes overproduction of erythroblasts, also causes increased destruction of erythrocytes. The evidence is not good for establishing a relationship between maternal conditions and the fetal state. In the case here noted, one twin was healthy; the other, erythroblastic. Nucleated reds appeared in the fetal circulation but not in the maternal. The condition may also, so writers say, be the expression of a general metabolic anomaly. This, of course, is begging the question. Nevertheless, it may be suggested that there is something of an analogy between this condition and leukemia. Leukemia is often considered a form of neoplastic overgrowth, in which the tissue concerned in the production of white blood cells, is affected. It seems that if that conception has any force, and we believe it has, then erythroblastosis may be looked upon also as belonging to the group of tumors, and in this case it is the erythroblastic tissue which is involved. If this should appear to be true, then the term fetal erythroblastomatosis would be significant.

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LABORATORY METHODS

A New Operating Table and Head Holder for Experimental Purposes*

By D. E. Jackson, M.D., St. Louis, Mo.

THIS table is made almost entirely of ordinary gas pipe and gas pipe fittings. It is intended that, in so far as possible, all the parts shall be of regular standard make and size and therefore easily obtainable. The table can be folded up completely as shown in Fig. 2. The table complete, including head holder and manometer stand weighs approximately fifty pounds. The frame, legs and braces (*c, d*) are made of half-inch pipe and fittings. The table is 35 inches high.

The wooden top (*a*) is 3 feet 8 inches long by 12 inches wide and 1 inch thick. The metal frame beneath this is 3 feet 5.5 inches long by 13.5 inches wide as measured from center to center of the pipes. The rear legs (*e, e*) pass upward and screw into side-elbows which are connected by close nipples with tees which are bored out large enough to turn loosely on the pipe which forms the rear end of the framework. The other openings of the side-elbows are connected by a short piece of pipe which holds the upper ends of the legs firmly in place. When the brace (*c*) is screwed out the rear legs are then thus free to rotate backwards and be folded completely back up over the top of the table. Two small notches are cut in the rear end of the top of the table to admit the revolving tees as the legs are folded back over the table top. Similarly the front legs (*f, f*) pass upward beneath the table but about 1.5 inches in front of the end of the wooden top. The legs here screw into side-elbows. These side-elbows are connected by a short piece of pipe which holds the upper ends of the legs firmly in position, while the other opening of each of the side-elbows (toward the table top) is connected (by a close nipple) to a tee which is bored out to turn easily around the pipe which forms the front end of the frame. When the brace (*d*) is screwed out the front legs are thus left free to fold backward up under the bottom of the table.

The pipe *l*, which is covered by a cap at the top, passes down and screws into a side-tee, the side opening of which is turned toward the table top and is connected by a close nipple with another side-tee, the side opening of which is turned down and carries a set screw to fasten these two side-tees and the pipe *l* in any position desired; for the straight opening of each of the two side-tees is bored out to slip easily over the two pipes which are located at the front end of the table, viz., the front end of the frame of the table and the pipe which connects the two side-elbows into which the upper end of the front legs are screwed. These two pipes are parallel and about 2.25 inches apart (from center to center) and the sliding of the two side-tees from side to side on these pipes permits the

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pipe *l*, and hence the head holder *k*, to be moved from side to side to any desired position.

The braces (*c* and *d*) are screwed into tees, the threads of one end of each brace being left-handed. The pieces *d*,′ *c*′ serve as handles for screwing in or out these braces. At the upper end the braces are screwed into the side open-

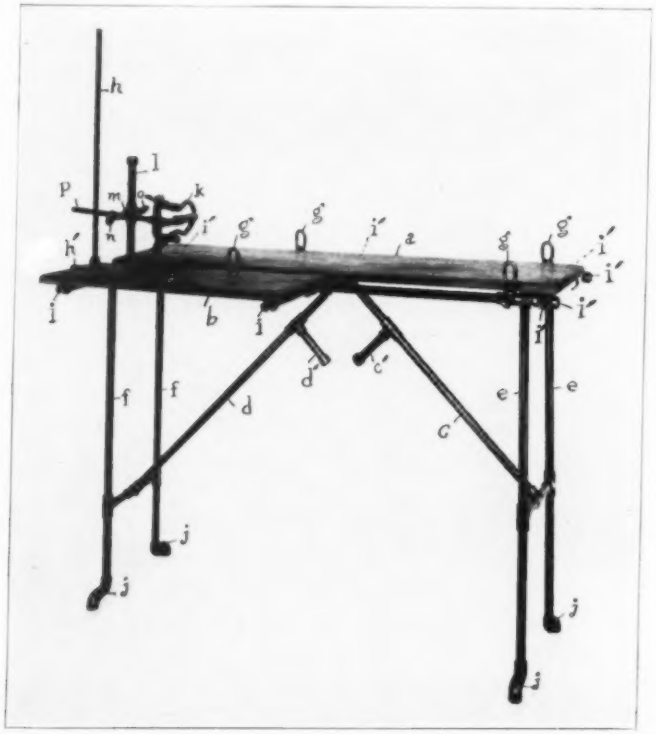

Fig. 1.—For description see text.

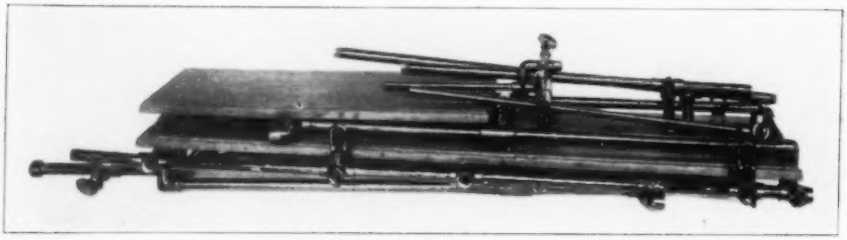

Fig. 2.—For description see text.

ings of a side-tee which is placed in the middle of the central cross pipe of the frame of the table.

The shelf (*b*), is movable and can be easily shifted to any one of four positions or left off entirely. Or a long shelf, the full length of the table, may be placed on one or both sides, or short shelves may be placed at one or both ends of the table. These various changes are made possible by screwing short

pieces of pipe (*i, i*) into the side openings in the frame of the table. There are ten of these openings, a part of which are indicated by the letter *i'*. The shelf (*b*) is held on to the short pipes (*i, i*) by small flat metal clamps such as are used to attach gas piping to walls, etc. The shelf can be quickly slipped off the pipes until the dissection is complete and then replaced to hold apparatus, etc.

At the floor each leg carries two elbows (*j, j, j, j*), joined together by a close nipple. This gives the table a wider base and prevents overturning. Castors may be attached if desired.

The loops (*g, g, g, g*) are used for the attachment of cords which hold the animal, and are half-inch size chandelier loops screwed into the upper openings of side-tees. The other openings (lateral) of the side-tees carry set screws. The straight opening of each of the side-tees is bored out to slide or turn easily on the side pipes of the frame. Thus by means of the set screws these loops may be quickly fastened at any desired position to fit various sized animals.

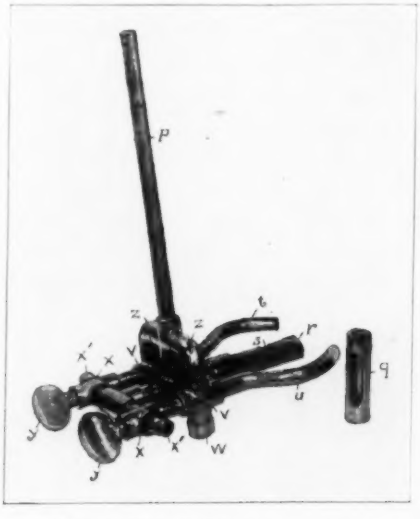

Fig. 3.—For description see text.

The pipe *h* (which holds the manometer, tambours, etc.) is quarter-inch sized piping and is 24 inches high. At the bottom it screws into the upper opening of a side-tee while the other (lateral) opening of the side-tee carries a set screw to fasten the side-tee (and hence the pipe *h*) in any desired position on the short pipe *h'*, which is screwed into one of the side openings (*i'*, not indicated at this place in the picture) of the frame. The straight opening of the side-tee is bored out so that it will slide or turn easily on the pipe *h'*.

At *m, n, o* in Fig. 1 is shown the method of attachment of the head holder (*k*). By means of the adjustments here indicated the head holder can be adjusted in any direction, laterally, up and down, back and forth, and it can be rotated entirely around or placed at any angle as is often desirable for operations on various parts of the head.

The head holder (*k*) (Fig. 3) can be entirely detached from the table and fastened on the animal out in the room. The head holder may also be

used on any ordinary table or operating board by simply attaching the pipe *l*, by means of a flange to the top of the table or board. The head holder is made of quarter-inch tubing and fittings in all parts except the piece *r* (Fig. 3) which is of half-inch piping. Near the center of this piece *r* is a hole one-half inch in diameter shown at *s*. This hole extends entirely through the piece *r*, and is for the purpose of admitting a stomach tube if one cares to administer a drug in this manner.

The piece *q*, is a wrench used to turn the various set screws about the table. It is a piece of half-inch pipe 3.5 inches long with a slot cut in one side.

The pieces *t* and *u* (Fig. 3) are 4.5 inches long and the curved part which passes around the animal's jaws is 3 inches long. Each of these curves should be wrapped with several layers of gauze which is tied on tightly by twine or soft copper wire. The animal's canine teeth are passed in front of the piece *r*, and then the pieces *t* and *u* are quickly closed down tightly on the outside of the jaws. The set screws *y*, *y* are quickly tightened and the animal is held secure, thus avoiding largely the use of a muzzle. The crosses *v*, *v* are bored out to turn freely on a 2.25 inch nipple which screws into the elbow *z*, and is capped by the cap *w*. The cross *x* is bored and filed out to slide freely back and forth on the short curved pipe *x'*. It is intended that this head holder should be quickly and effectively fastened on to the animal and that the pipe *p* may be used as a handle to carry out any further manipulations of the animal.

Any ordinary plumbing or gas fitting establishment can readily and cheaply furnish all the metal parts of this table and head holder. The wood top and shelf (or shelves) can be obtained in most places without difficulty.

Some of the ideas incorporated in this table have been suggested by Mr. John A. Higgins.

The Journal of Laboratory and Clinical Medicine

VOL. I. FEBRUARY, 1916 No. 5

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Ann Arbor, Mich.

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EDITORIALS

The Measurement of the Mass Movement of the Blood

THE object of the circulation is to supply the tissues with blood, and the adequacy of the supply will depend mainly on the difference of pressure between artery and vein and the calibre of the intervening vessels. It is impossible, from the blood pressure measurements alone, to form any accurate estimate of the rate at which blood is flowing through an organ or part (the so-called mass movement of the blood). It often happens, for example, that perfectly normal blood pressure values may be obtained from the vessels of a part (such as the hands or feet) the blood supply of which is nevertheless obviously either above or below the normal as indicated by the subjective sensations of heat or cold; thus, cold hands or feet may be complained of when blood pressure measurements in the artery and vein give normal values. This fact suggests a method for measuring the real blood supply, namely, to ascertain the amount of heat which the part gives off. Such a method has been worked out by G. N. Stewart, who has used it in the study of a series of clinical cases with results which cannot but be of importance and value in the clinic.

Indeed, Stewart has been able in many cases to detect changes in blood-flow where no such change was suspected, and by repeating the measurement from day to day, in cases where the blood-flow was found to be inadequate, he has been able to supply information without which it would have been im-

possible to decide on the correct line of treatment. We will go into some of these cases in greater detail later on, but meanwhile it may be of interest to point out *the general principles of the method.*

The apparatus consists essentially of a vessel containing a known quantity (3,000 c.c.) of water and a thermometer from which a change of temperature of a hundredth of a degree Centigrade can be read. In order to diminish as much as possible the loss of heat between the vessel and the outside air, the walls of the vessel are double, the space between being stuffed with broken cork. The top of the vessel is closed with a thick cork plate, having suitable openings in it for the hand or foot and for the thermometer and a stirrer (feather) with which to keep the water in constant motion. The apparatus is called a *calorimeter.*

After the hand or foot has been in the calorimeter, with the water a few degrees below that of the body, for a certain time, the temperature of the water will, of course, become raised and the degree to which this occurs, multiplied by the volume of the water in cubic centimeters, will give in calories the amount of heat dissipated. By the application of a very simple formula it is now an easy matter to calculate how much blood must have passed through the blood vessels of the part in order to give out the observed amount of heat; for, if *we divide the calories* by the *difference in temperature* between the inflowing and outflowing blood of the part, the result must indicate *the volume of blood,* in cubic centimeters, that has passed through it. Thus, if Q equal the amount of blood, H, the calories of heat given out to the calorimeter, T, the temperature of the arterial blood, and T′, the temperature of the venous blood, then we have the equation: $Q=\frac{H}{T-T'}$. The only other point in this equation that we need dwell on at present is the measurement of the temperature of inflowing and outflowing blood. It has been shown by Stewart that the temperature of the inflowing blood is the same as that of the mouth, or 0.5° C. below that of the rectum, and that the temperature of the outflowing blood equals the average temperature of the water in the calorimeter during the observation. To allow for the specific heat of blood, the result is multiplied by $\frac{10}{9}$.

Theoretically, then, the method is very simple, and there are no unusual technical difficulties in carrying it out. The only special precaution is that the air surrounding the calorimeter should be kept tolerably constant in temperature, so that we may be enabled to allow in our calculations for the loss of heat from the calorimeter itself, this value being obtained by observing the change of temperature in the calorimeter for a certain period of time after the hand has been removed from it. The observation should, therefore, be made in a small room with closed doors, or if it must be made in a ward, the space around the bed should be well surrounded with curtains.

The results are calculated on the basis of grammes of blood flowing through 100 c.c. of tissue in one minute. In a normal, healthy individual the average flow in the hand is from 12 to 13 gms. for the right hand, and about half a gramme less for the left. This difference between the two hands corresponds, of course, with their relative degree of development. The average foot-flow is much less, and varies according to whether the patient is sitting up or lying

down while the measurement is being made. In a normal individual, while lying down, it was 5.11 gms. in the right foot and 5.23 gms. in the left, per 100 c.c. of foot; but only 3.96 gms. for the right, and 4.1 gms. for the left foot, while sitting up. The results have been found to undergo only a slight variation from month to month in a given healthy individual, provided the air temperature during the different observations is the same. To obtain these constant results, however, it is very important to have the person long enough in the room before the observations are begun, especially if he be a dispensary patient and has been in the open air with bare hands. Approximately standard conditions of room temperature must become established if comparison of flow in the same patient at different times or of different patients is proposed. Finally, it should be remembered that, although the flow under standard conditions is very constant for each individual from time to time, yet it varies in different individuals, both with regard to the absolute amount of the flow and the ratio between the two hands or feet. When the total flow in the two hands is compared with that in the two feet, it is usually found that a ratio of about 3 to 1 is obtained.

Before proceeding with the results obtained in clinical cases, it may be of interest to consider some of the physiological causes for variations in blood flow. As above indicated, the most marked of these is probably *the temperature of the room. The temperature of the water in the calorimeter* has likewise a great influence, and for the comparison of different cases it is always important that the room and calorimeter temperatures be stated alongside the results. *Muscular contractions,* produced by moving the fingers in the calorimeter, were found to produce an enormous change in flow; thus, with calorimeter temperatures of 24.38° C. and 23.64° C. for the right and left hands respectively, and a room temperature of 21° C., the flow was 15.4 gms. per 100 c.c. per minute of the right hand while it was being made to contract in the calorimeter, and only 4.9 gms. for the left, which remained at rest. Since 4.9 gms. is a much smaller flow than was ever found in the hand of this person with both hands at rest, the increased flow in the contracting hand was evidently accompanied by a diminished flow in the hand that was at rest. Moderate *constriction of the arm* so as to obstruct the venous circulation caused a great diminution of flow, which however increased later because the blood began to pass the constriction, on account of the rise in venous pressure. By increasing the constriction of the arm up to and beyond the systolic pressure, as estimated by the clinical method, no blood-flow occurred, and as the pressure was gradually reduced below the systolic, the flow only became slightly increased for a considerable decrement of pressure. This result indicates that the pressure in the armlet must fall somewhat below the diastolic pressure before any marked increase in the flow through the hand occurs.

The most interesting physiological condition that causes changes in the blood flow is a *vascular reflex* set up by immersing the opposite hand or foot in hot or cold water. In the former case the blood-flow through the observed hand increases, while in the latter it decreases. This change may be of a temporary character, or it may persist throughout the whole period of immersion of the hand. Constriction elicited by cold seems to be more durable in the foot

than the hand, and this fact must be borne in mind when making foot measurements. The practical importance of having a reflex which we can observe quantitatively, is, of course, very great, for it permits us to study the effect of lesions affecting either the nerve or the nerve center concerned in vascular reflexes.

The reliability of the method was evidenced by the results obtained in *clinical cases in which obvious differences existed* in the blood-flow of the two hands or feet, such, for example, as in a case with a recent cicatrix of one arm, and in one in which one of the arm nerves had been severed. The most interesting point observed in these cases was that the vascular reflex did not behave in the normal fashion, being either greatly exaggerated or depressed. The vasomotor control of the new blood vessels of the cicatrix, or of the new nerve fibers in the regenerated nerve, was evidently very unstable. Several cases were observed where the venous return from the hand was interfered with by a tumor. Marked diminution of blood-flow was observed, which was not the case, however, in other cases where edema existed as a result of lymphatic obstruction. The value of the method in diagnosing the edema due to lymphatic or venous obstruction is thus illustrated. In a case of inflammatory redness and swelling of the hand as the result of an infection, a considerable increase of blood-flow was observed, but, still more interesting, a great decrease in the flow in the normal hand also occurred. The suggestion is made that this curtailment of flow in the normal hand occurs for the purpose of providing an increased flow in the infected area. In other words, in order to provide for an increased flow in the infected area, a vasoconstriction, possibly elicited reflexly through the pain nerves of the infected hand, is brought about elsewhere and particularly in the symmetrically placed part of the opposite side. Furthermore, it was observed, in this infected case, that the immersion of the opposite hand in cold water caused scarcely any reduction of the flow in the infected hand. The vasomotor impulses therefore for some reason had become blocked. In other cases of nonbacterial inflammation of the hand, as in gout, no sign of vasoconstriction block was obtained.

We will now pass in review certain groups of clinical cases in which a change in blood-flow might not be detected by the employment of ordinary clinical methods.

Anemia.—The blood-flow in the hand was found to be smaller than normal in cases of pernicious anemia, chlorosis and secondary anemia. The deficiency was less, however, in the chlorotic group than in the other cases of anemia. Sometimes the degree of deficiency was very marked; thus, in a case of marked secondary anemia, the flow was only 1.26 gms. per 100 c.c. of hand for the right, and 1.18 gms. for the left hand. Since it has been shown by Plesch that in anemia the minute volume of the heart is increased, it must follow that the vasoconstriction at the periphery will compel more blood to pass through the lungs; in other words, that it is a compensatory arrangement for increasing the flow through the lungs, so as to make up for the deficiency of blood.

Fever.—Since changes in the cutaneous circulation probably constitute the chief factor in the derangement of the temperature-regulating mechanism in

fever, it is evidently of great advantage to be able to measure such changes quantitatively. This has been done by Stewart in several cases of typhoid fever and in one case of pneumonia. The general result has been that the flow in the feet never exceeded the normal flow, and was usually much below it. This tendency to vasoconstriction seems to be carried into convalescence. For practical reasons the hand-flow has not been so extensively studied. This hyperexcitability of the vasoconstrictor mechanism at the periphery is most naturally interpreted as a defensive reaction of the organism by which an increased supply of blood is imported to those internal organs which bear the brunt of the infection. When we consider that in spite of this constriction at the periphery the blood pressure is low and the pulse dicrotic, we must conclude that there is considerable dilatation of other vascular parts, especially the splanchnic area. A very practical application of these facts presents itself in considering the rationale of the cold bath treatment for fever. If, for example, we conclude that the cutaneous constriction is in the interests of an increase in the blood-flow to the organ on which the stress of the infection falls, it will evidently be more rational to lower the temperature by methods which will not diminish, and may even increase, the cutaneous constriction than to do so by causing the vessels to dilate. In other words, the use of antipyretics seems to be contraindicated, since they diminish the body temperature, causing vasodilatation at the periphery with a consequent withdrawal of blood from the seat of infection. Such drugs "diminish the temperature, it is true, but at the cost of defeating the beneficial redistribution of the blood, which it is the function of the peripheral vasoconstriction to ensure."

Cardio-vascular Diseases.—Taking all the observations that have so far been made on *cardiac cases,* certain general conclusions, which are only provisional in some instances, may be drawn. The hand-flow is far more apt to be markedly deficient in cases where there is evidence of serious impairment of the myocardium than in cases where gross valvular lesion exists while the heart action is strong and orderly. This indicates that it is more serious for the pumping force of the heart to be interfered with than for its valves to be leaky. No matter how tight the valves may be, the output will be small and irregular if the stroke of the pump is feeble. This general statement applies more particularly in the case of mitral than of aortic insufficiency. Even where there is considerable venous engorgement, the flow may be little diminished, provided the myocardium is not impaired, and a failure of the myocardium will immediately be indicated when a marked diminution in the hand-flow occurs with great engorgement of the veins. Stewart and R. W. Scott have recently reported observations on cases of auricular fibrillation treated with digitalis. In untreated cases the blood-flow was subnormal. In three patients the blood-flow was promptly and decidedly increased after the administration of digitalis, but in a fourth case, which had not been considered to respond well to this drug, the blood flow was somewhat increased when the drug was stopped after a long course of it. The general conclusion is that in cases of fibrillation, which are evidently benefited by digitalis, the blood-flow through the hands becomes coincidently increased.

As would be expected, *arteriosclerosis* was found to be associated with a small blood-flow, and the vasomotor reflexes to be weaker than in normal per-

sons. In *thoracic (aortic) aneurysm* the flows were either of the normal order of magnitude or at least not so conspicuously insufficient as might be expected. Of course where the aneurysm was of such a size as to cause pressure on the subclavian artery or vein, a diminution in flow of the corresponding hand was observed, but otherwise aortic aneurysm in itself, although it may cause great changes in the character of the pulse beat, does not evidently affect the mass movement of the blood. In a case of aneurysm of the subclavian artery, more recently examined, the blood-flow was much greater in the corresponding hand (12.52 grams per 100 c.c. of hand per minute) than in the opposite hand (6.36 grams). Special interest attaches to the case because the amplitude of the pulse was very obviously *less* on the affected side than on the sound side, and, judging from this alone, misleading conclusions regarding the mass movement of the blood might have been drawn. The difference between the systolic and diastolic pressures (the pressure pulse) was found to be much less on the affected side (*S.* 110, *d.* 88 mm. Hg.) than on the normal (*S.* 130, *d.* 70 mm. Hg.). By clinical blood pressure measurements, therefore, false estimates of blood-flow are quite likely to be drawn.

The increased blood-flow was no doubt due partly to vasodilatation brought about by pressure of the aneurysm on the brachial plexus and partly to the lower resistance to the flow of blood into the dilated right subclavian. The pressure on the plexus interfered not only with the tonic vasoconstrictor impulses but also with those to the skeletal muscles, for there was marked loss of power as well as pain in the affected arm. Eleven days after ligation of the right innominate the flow in the corresponding hand had of course diminished (to 5.44 grams), but the point of particular interest is that the flow in the opposite hand had reciprocally increased. "The cutting off of the path through the innominate and right common carotid obviously permitted more blood to enter the alternative route of the left subclavian and left carotid."

The gradual development of a collateral circulation could be very accurately followed on the operated side; in 17 days after the operation it was 4.76 grams, in 24 days it was 4.86 (cool morning), and in 31 days, by which time the hand was being freely used, it was 8.55 grams. Even with this reestablishment of adequate flow no pulse was palpable on the right side.

Interesting observations were also made on cases of *Raynaud's disease*. As would be expected, the flow was small, the diminution being more or less proportional to the duration of the disease. The contralateral vasomotor reaction to cold was also peculiarly intense. In diabetic gangrene of the feet a very subnormal flow was also observed in both the hands and the feet. The vasomotor reflexes were also feeble.

Besides cardiovascular diseases inequality in flow in the two hands may be due to *nervous conditions*, and a recent paper deals in detail with the characteristics by which the exact cause of the inequality may be diagnosed. When due to mechanical causes, the inequality remains unchanged over short periods of time, whereas in nervous conditions the inequality changes from day to day. Of course, over longer periods the mechanical inequalities may also change, as, for example, in cases of thrombosis or embolism. Another important distinction is brought out by testing the general vasomotor reflexes by changing the temperature of the room and observing whether the ratio between the two hands

remains unchanged or becomes altered. If it becomes altered, the inequality must be due to nervous causes. As a practical method of testing these cases, Stewart suggests that the blood-flow measurement should be made at intervals of several hours apart on the same day with different room and calorimeter temperatures. As illustrating the importance of these facts, Stewart details the results obtained in a case of embolism and thrombosis involving the left arm and right leg. The ratio in the hands was 1:2.7. By repeated examination it was found that the ratio between the two feet became progressively larger, until at last it mounted to 1:9.28, thus indicating a tendency to gangrene in the foot with the curtailed flow. The interesting point in this case, however, was the fact that when the ratios of the sum of the foot and hand flows were compared over a period of four months, they were found to be practically constant, namely, 1:1.90. That is to say, even though one foot had become much less vascular than the other, the ratio of blood flowing to the hands and feet was maintained at its usual level, thus showing that the blocking of the vascular path to one leg was associated with a reciprocal dilatation of the path to the other leg, so that the normal partition of the blood between the legs and the rest of the body was scarcely disturbed. This indicates that the blood which normally finds its way through the two common iliacs seems eventually, when the main part of the path from the one common iliac is blocked, still to find its way through the one which remains pervious, room being made for the additional quantity of blood by vasodilatation. It is further suggested that this adjustment occurs in such cases so that there may be no interference with the elimination of heat by way of the extremities. A certain amount of cooling has to be effected through the extremities, and if the circulation through one of these should be curtailed, then that through the other must become reciprocally increased. This adjustment in chronic cases would not probably be evident when acute curtailment of the blood-supply to one limb is produced by ligation or amputation.

An interesting case given in detail is the one of *hematoma in the axilla.* The hematoma was due to a gunshot wound. The pulse was distinctly less on the injured side, and nutritional changes in the hand raised the question as to whether there existed a degeneration of branches of the brachial plexus, or whether the flow was merely mechanical, due to pressure of the hematoma on the artery or vein. By testing the vasomotor reflexes it was found that the cause was mechanical and not due to vasoconstriction. It was also important to decide in this case whether the curtailment of flow had become so marked as to indicate surgical interference, in order to prevent the onset of gangrene. It was found that there was enough blood in the hands (5.32 gm. per 100 c.c. per minute for the right, with a ratio of 1:2.77). Five days later the ratio was unchanged, so that the cause could scarcely have been vasomotor. One hundred and ten days later the right hand had risen to 9.3 gm. with a ratio of 1:1.24, indicating that great improvement in the blood-flow had taken place. Without the information furnished by the blood-flow measurements it would have been impossible to tell by palpation of the pulse whether in this case surgical interference was justified.

Diseases of the Nervous System.—The effect of *neuritis* on the flow varies with the duration of the case. In cases of early peripheral unilateral neuritis

there was an increase of flow bringing the ratio between the two hands to 1:1.34, with the greater flow on the diseased side. Though the vasoconstrictor reflexes were as great as normal, yet they were evanescent in character. These results are interpreted as being due to a partial paralysis of the vasoconstrictor fibers in the inflamed nerve. Similar vasoconstrictor paralysis, as we have seen, may also be caused by pressure of an aneurysm on the brachial plexus. In neuritis of long standing the flow was cut down, the ratio becoming 1:1.43, with the greater flow on the healthy side. The changes here are probably due to anatomical alterations in the lumen of the tube, perhaps a thickening of the intima, as had previously been suggested by Wingate Todd. In a case of *motor neurone disease* without any involvement of the sensory skin nerves the flow was normal, and the reflexes well-marked. The conclusion is that involvement of the muscle nerve does not interfere with blood-flow to anything like the same degree as does involvement of the skin nerve, the blood-flow through the skin being relatively more important than that through the muscles.

Hemiplegia.—A deficiency of blood-flow of the paralyzed side, varying however in degree, was usually observed, and the ratio may be 1:1.4 in favor of the healthy side. The vasomotor reflexes were found to be altered, the most usual change being that vasoconstriction was more easily produced than vasodilatation; indeed, in some cases there was observed an abnormal tendency to vasoconstriction.

Tabes dorsalis.—The flow was distinctly diminished, especially in the feet, although also in the hands, and the vasomotor reflexes were also quite feeble. Sometimes there was inequality in the flow of the two hands, which however need not necessarily indicate a unilateral lesion of the cord in the cervical region. In the discussion of these cases Stewart points to the value of the method in the detection of *malingering.* He cites the case of a patient in whom malingering was suspected, but in whom an examination of blood-flow revealed so small a foot-flow as to make it very probable that an actual pathological condition existed. Total absence of the vasomotor reflexes confirmed the diagnosis of tabes dorsalis as did also the further progress of the case.

—*J. J. R. M.*

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Concentration of Protective or Curative Bodies in Antisera

PASSIVE immunity, which may be of great importance in the prevention or treatment of disease, depends on the fact that the serum of an animal that has been actively immunized against a given microorganism contains certain principles which are able to protect another animal that is given an injection of this immune serum. This principle, first demonstrated by Richet and Hericourt in 1888, became at once of extreme importance following the experiments of von Behring and Kitasato who demonstrated that the serum of rabbits that had been immunized with diphtheria toxin contains a substance that neutralizes that toxin and thereby cures animals that are suffering from the effect of such toxin. In the sera of animals immunized against other bacteria or bacterial products, it has since been shown that various immune bodies other than antitoxins are present, such as lysins, agglutinins, tropins, and the like. These latter substances have an affinity for, as well as a harmful action against, the various microorganisms that have been employed to produce them, and in many instances give great therapeutic results. It is probable that failure to produce better results is due in a large part to the failure to understand the best method of producing these antisera, and particularly to the failure to appreciate the best method of administering them. This becomes evident when we consider the very striking results that have been obtained with at least one such serum—antimeningitis serum—described by Wassermann and particularly utilized by Flexner who obtained remarkable results following the intraspinal injection of the serum in epidemic meningitis.

One of the important lines of future progress in the employment of therapeutic immune sera lies in the methods of concentrating the active principles of the serum. The antitoxins, antimeningitis serum, and the like, are produced by immunizing large animals with the filtrates or cultures of the bacteria that they are to be used against, but it is often necessary to employ relatively large doses of the serum of such immunized animals to produce the desired results. Antisera of this type being derived usually from the horse are not entirely without untoward or even dangerous effects when injected into human beings, because they are foreign proteins and we know that the body reacts in certain definite ways to the injection of foreign proteins. Foreign proteins, when they are not split in the intestinal canal for further assimilation, but injected parenterally, may give rise to delayed symptoms, such as pains in the joints and an urticarial eruption. A second injection of such a foreign protein may give rise to an immediate reaction, which is known as anaphylactic shock, characterized by respiratory symptoms, and in some cases by sudden death.

The concentration and purification of these immune sera, then, would bring about two desirable results. It will first of all remove a large percentage of those proteins in the foreign serum which do not contain the antibodies, and secondly will concentrate those remaining proteins which are found to contain the active therapeutic principles that it is desired to utilize. The Gibson method of concentrating antitoxins is a chemical means of producing these desiderata. This method, which is currently employed for concentrating diphtheria antitoxin, consists in precipitating out the globules of antitoxic serum from the horse by means of one-half saturation with ammonium sulphate and further re-

moving the globulins that are not soluble in a saturated solution of sodium chloride which leaves behind the globulins that contain practically all the antitoxins. Antitoxins prepared in this manner may be greatly concentrated so that one cubic centimeter will contain many times the number of antitoxic units that are present in one cubic centimeter of the original serum, and the antitoxic globulins prepared in this way furthermore do not seem to contain the irritative substances of the horse serum which give rise to urticaria.

It was further shown, however, by Banzhaff and Gibson[1] that the globulins are increased during immunization of horses against diphtheria toxin and they have found that the globulins which contain the antitoxin rise from 60 to 90 per cent of the total globulins present. So in employing the concentrated serum, although one avoids the injection of the total amount of protein in the corresponding original serum, yet a relatively large amount of foreign globulin is introduced.

Another method of concentrating the antibodies of immune serum might be devised by using the specific precipitin reaction. This reaction, as is well known, depends on a peculiar property that an immune serum derived from immunizing animals with bacteria, or with soluble foreign protein substances, contains a body known as a precipitin which, when the serum is mixed with antigen, gives rise to turbidity, floccillation and the formation of a precipitate. This test is most frequently employed in medico-legal tests for human blood where it has proved most valuable. It was originally suggested by Kraus in a combination of bacterial extract and its corresponding immune serum. Considering the question of an immune serum derived from the horse, we may produce a precipitate either by adding an extract of the bacterial substance that had given rise to this serum to it, or by adding to such a serum the serum of another animal, let us say a rabbit, that had been immunized against horse serum. In the first of these cases the immune serum acts as a precipitin, namely, when the precipitinogen is added in the form of a bacterial extract; in the other case the horse serum acts as the precipitinogen. In both cases a precipitate is produced, but the amount of horse serum necessary to produce the maximum precipitate varies greatly in the two types of reaction. When the immune sera acts as a precipitin it may be added in excess, but when the immune sera is the precipitinogen it must be added in relatively dilute form to produce the maximum precipitate.

That reactions of this type may be of practical importance in concentrating the curative properties of immune sera was first shown by Dehne and Hamburger[2] who added rabbit antiserum to antitoxins from the horse and found that the antitoxin was brought down in the precipitate thus formed. These experiments were made with tetanus antitoxin and similar ones with diphtheria antitoxins by Atkinson and Benzhaff.[3] Such results, while of great theoretical interest, are not of practical significance in concentrating antitoxins, owing to the fact that the antitoxins are brought down only when great excess of rabbit antihorse serum is added, which would preclude the practical use of this method for the concentration of large amounts of horse serum.

The reverse of this reaction has been shown to be possible in at least one combination, by the work of Gay and Chickering[4] who found that those substances which protect animals against infection by means of pneumococcus pres-

ent in the sera of horses immunized against this organism may be concentrated in the precipitate produced by adding an extract of pneumococcus to this horse-antipneumococcus serum. In such a reaction, as will be seen, the component necessary in excess is the immune serum (precipitin) and for this reason large amounts of such immune serum can be completely precipitated by adding relatively small quantities of the extract of pneumococcus. These precipitates are found in the experiments of Gay and Chickering and later by Chickering,[5] to contain practically all the protective antibodies of the original serum, and moreover to contain relatively small amounts of protein as compared with the original serum, often as little as 1/30 or 1/50 of the protein of the original serum in aliquot parts. It is evident that these protective bodies are simply adherent in some mechanical way to the precipitate for they can be leached out from the precipitate by dilute sodium carbonate at 42°. This relatively pure antibody solution of low protein content is now being tried in the place of the original serum in the treatment of some cases of pneumonia with apparent promise of as good results as those obtained with the original serum.

These observations on the concentration of antibodies in pneumococcus serum naturally led the author to further studies of other antibodies of various sorts. The first report on these studies will shortly appear in the *Journal of Immunology*, but it may be stated that they have, so far, been rather discouraging and it would seem that the case with pneumococcus serum is the exception rather than an example of a general law. It may well be, however, that the method employed with other types of antiserum has not been the one best adapted to produce the results desired. At least other attempts are indicated in this field which may lead not only to practical results but to results of great theoretical interest when one considers the possibility of studying antibodies in perhaps the purest form in which they have been obtained.

—*F. P. G.*

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Diphtheria Carriers

A RECENT investigation of diphtheria carriers in Detroit is reported by Goldberger, Williams and Hachtel, in Bulletin No. 101, of the Hygienic Laboratories, of the United States Public Health Service. The problem of diphtheria carriers has become one of considerable importance and has been given special prominence of recent years by the studies of von Scholly, Moss, and Nuttall and Graham Smith. The writers of the report mentioned above studied 4,093 people in the city of Detroit, and found that 0.928 per cent harbored bacil-

li identical morphologically with the Klebs-Loeffler bacillus. This figure is rather lower than those of some other investigators, but would indicate, as stated by the writers, that there were from 5,000 to 6,000 diphtheria carriers in the city of Detroit.

Of 19 cultures isolated from 19 of the carriers, only two were virulent, which would indicate that only 0.097 per cent of the people examined carried organisms capable of producing disease. An interesting further point is that the bacillus Hoffmanii was present in at least 41.9 per cent of over 2,000 individuals examined, and that the 49 cultures morphologically identified as bacillus Hoffmanii were avirulent. This would confirm the impression gained, we believe, by most experienced laboratory workers, that a true Hoffmanii can be distinguished with considerable certainty from a Klebs-Loeffler bacillus by morphological examination alone, and that its significance is probably that of a frequently present saprophyte of the throat and pharynx. The studies of Goldberger, Williams and Hachtel also indicate that in examining for diphtheria carriers, it is best not to confine oneself either to the nose or throat, but that cultures should be taken from both places in every case.

—*H. Z.*

Erratum

IN the January issue of the Journal in the article by Dr. Roger S. Morris on "Methods for the Determination of Glucose in the Blood" the first sentence on page 254—"For example, 21 c.c. of blood filtrate, representing 3 c.c. of blood, gave a titration of 5.5 c.c. $\frac{N}{10}$ permanganate," should read as follows: "For example, 21 c.c. of blood filtrate, representing 3 c.c. of blood, gave a titration of 5.5 c.c. $\frac{N}{50}$ permanganate."